

1 Student-led original research article to *Oecologia* (population ecology)

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3 ***The effects of warming on the ecophysiology of two***
4 ***co-existing kelp species with contrasting distributions***

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14 ***Student-led highlighted paper:*** *Through a combination of field surveys and a manipulative*
15 *experiment with multiple ecophysiological response variables, we show that current and*
16 *predicted future thermal maxima induce sublethal effects in a cold-water habitat-forming*
17 *kelp species, which will likely lead to a continued range contraction. We show that loss of*
18 *marginal populations will be primarily driven by ecophysiological responses to high*

19 *temperatures, and although compensatory mechanisms may reduce top-down pressure on*
20 *marginal populations, this is unlikely to be important within the biogeographical context*
21 *examined. The work is novel and topical because it disentangles the relative importance of*
22 *physiological and ecological processes which underpin range shifts in a marine foundation*
23 *species.*

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25 **Author contributions:** All authors conceived and designed the experiments. MH performed
26 the experiments. MH and AF analyzed the data. AP provided and analysed field-based
27 survey data. MH and DS wrote the manuscript; AF and AP provided editorial advice.

28

29

30 **Abstract**

31 The northeast Atlantic has warmed significantly since the early 1980s, leading to shifts in
32 species distributions and changes in the structure and functioning of communities and
33 ecosystems. This study investigated the effects of increased temperature on two co-existing
34 habitat-forming kelps; *Laminaria digitata*, a northern boreal species and *Laminaria*
35 *ochroleuca*, a southern Lusitanian species, to shed light on mechanisms underpinning
36 responses of trailing and leading edge populations to warming. Kelp sporophytes collected
37 from southwest United Kingdom were maintained under 3 treatments; ambient temperature
38 (12°C), +3°C (15°C) and +6°C (18°C) for 16 days. At higher temperatures, *L. digitata* showed a
39 decline in growth rates and Fv/Fm, an increase in chemical defence production and a decrease
40 in palatability. In contrast, *L. ochroleuca* demonstrated superior growth and photosynthesis
41 at temperatures higher than current ambient levels, and was more heavily grazed. While the
42 observed decreased palatability of *L. digitata* held at higher temperatures could reduce top-
43 down pressure on marginal populations, field observations of grazer densities suggest that
44 this may be unimportant within the study system. Overall, our study suggests that shifts in
45 trailing edge populations will be primarily driven by ecophysiological responses to high
46 temperatures experienced during current and predicted thermal maxima, and although
47 compensatory mechanisms may reduce top-down pressure on marginal populations, this is
48 unlikely to be important within the current biogeographical context. Better understanding of
49 the mechanisms underpinning climate-driven range shifts is important for habitat-forming
50 species like kelps, which provide organic matter, create biogenic structure and alter
51 environmental conditions for associated communities.

52 **Keywords:** Ocean warming, macroalgae, chemical defence, thermal tolerance, range shifts

54 **Introduction**

55 Anthropogenic climate change is a major threat to the integrity and persistence of marine
56 biodiversity and ecosystems (Hoegh-Guldberg and Bruno 2010). Recent increases in sea
57 temperature have driven shifts in species' distributions and consequent changes in the
58 structure and functioning of communities and ecosystems (Pinsky et al. 2013; Poloczanska
59 et al. 2013; Wernberg et al. 2016). Globally, sea surface temperatures have increased at a
60 rate of $>0.1^{\circ}\text{C}$ per decade since the mid-20th century (IPCC 2013). In the northeast Atlantic,
61 temperatures have increased from around $0.3\text{-}0.6^{\circ}\text{C}$ per decade since the 1980s, with 2000-
62 2010 being the warmest decade recorded in this region (Belkin 2009; Hughes et al. 2010;
63 Smyth et al. 2010). In addition to decadal-scale gradual warming trends, the importance of
64 short-term extreme warming events (e.g. 'marine heatwaves') in structuring marine
65 ecosystems is rapidly gaining recognition (Hobday et al. 2016) and the number of extremely
66 hot days (in terms of sea temperatures) has increased along most of the global coastline in
67 recent decades (Lima and Wethey 2012). Both gradual warming and discrete warming
68 events can invoke physiological stress when thermal thresholds are exceeded, which in turn
69 may impact individuals, populations and communities (Smale and Wernberg 2013;
70 Wernberg et al. 2016).

71 Kelps (members of the order Laminariales) are large conspicuous brown macroalgae with
72 complex tissues and generally high biomass (De Wreede and Klinger 1988). Kelp forests,
73 formed by aggregations of canopy-forming kelp sporophytes, represent one of the most
74 productive ecosystems globally and generally support highly diverse and abundant
75 communities in temperate shallow seas (Steneck et al. 2002). Kelps also provide a suite of
76 ecosystem services including biogenic storm protection, nutrient recycling, natural

77 resources, and nursery grounds and habitat for commercially important fish and crustacean
78 species (Beaumont et al. 2008; Bertocci et al. 2015; Smale et al. 2013). As kelps are generally
79 cold-water adapted species, their geographical distributions, specifically the equatorial
80 range edge, are strongly controlled by temperature (Lüning 1984), although other factors
81 such as light and nutrient levels are important in determining species distributions and kelp
82 forest structure (Desmond et al. 2015; Gorman et al. 2013; Smale et al. 2016). As such,
83 oceanic warming has been linked to loss of marginal populations, range contractions and
84 significant reductions in kelp forest extent (Tuya et al. 2012; Wernberg et al. 2013).

85 Conversely, rising seawater temperatures have also been linked to range expansions at the
86 poleward edge for some warmer water species, such as *Laminaria ochroleuca* (Smale et al.
87 2015). It is evident that recent changes in ocean climate have influenced the structure and
88 extent of kelp forests in some temperate regions, and further changes are predicted to
89 occur (Brodie et al. 2014; Müller et al. 2009; Wernberg et al. 2016).

90 Along the southwestern coasts of the United Kingdom (UK) a number of different kelp
91 species co-exist (Smale et al. 2013), including more northerly-distributed species (e.g. *L.*
92 *digitata*, *Alaria esculenta*), more southerly-distributed species (e.g. *L. ochroleuca*, *Saccorhiza*
93 *polyschides*) and non-native species (*Undaria pinnatifida*). As a result, the structure of kelp
94 canopies is complex, with high spatiotemporal variability, and the relative abundance of
95 different species is strongly influenced by biotic and abiotic processes (Smale et al. 2013).

96 On moderately wave-exposed shores, the ‘cold water’ species *L. digitata* may co-exist with
97 ‘warm’ water kelp *L. ochroleuca* in the lower intertidal and immediate subtidal zones. Whilst
98 *L. digitata* is a more northerly-distributed species, with a range that extends from the Arctic
99 to southern Brittany, *L. ochroleuca* is a more southerly-distributed species of Lusitanian

100 origin, which extends from the southwest of the UK into the warm waters off Morocco and
101 into the Mediterranean (Fig. 1, Smale et al. 2013). Emerging evidence suggests that the
102 southernmost populations of *L. digitata*, located in Northern France, have diminished in
103 recent decades leading to a poleward contraction of the trailing range edge (Raybaud et al.
104 2013). On the other hand, marginal populations of *L. ochroleuca* have proliferated at the
105 leading range edge, in line with recent warming trends (Smale et al. 2015). The co-existence
106 of both a climate change 'winner' and 'loser' presents an interesting model system for
107 examining processes acting at both the trailing and leading range edges, in order to improve
108 understanding of mechanisms underpinning species' distributions (Bates et al. 2014). Here,
109 we examined the effects of thermal stress on the ecophysiological performance of these
110 contrasting kelp species in the southwest UK by assessing a number of factors, direct and
111 indirect, that may be affected by increasing temperature.

112 Individual response variables, such as growth and fecundity, are controlled by biochemical
113 and physiological processes that are strongly influenced by temperature. As such, thermal
114 stress has a direct effect on these responses, especially when thermal thresholds are
115 exceeded and available resources are allocated to maintenance, for example (Andersen et
116 al. 2013; Bolton and Lüning 1982). An example of an indirect effect of thermal stress on
117 macroalgal performance is the influence of increased temperature on plant-grazer
118 interactions (O'Connor 2009). Macroalgal success at the individual and population levels can
119 be limited by herbivory, which in turn may be mitigated by the capacity of a primary
120 producer to defend itself against grazers (Herms and Mattson 1992). The purpose of
121 chemical defence is to maintain algal fitness by interfering with a herbivore's ability to graze
122 successfully. Effecting chemical defence against herbivory is beneficial, as intense grazing

123 will reduce a plant's fitness (Bigger and Marvier 1998) and can ultimately be fatal (Scheibling
124 et al. 1999). Plant-grazer interactions in marine ecosystems are complex and represent a key
125 biotic factor influencing entire communities (Bigger and Marvier 1998), particularly within
126 kelp forests (Ling 2008; Norderhaug and Christie 2009; O'Brien and Scheibling 2016; Vergés
127 et al. 2014). Phaeophytes produce a range of primarily carbon-rich and therefore
128 metabolically costly defensive compounds including polyphenolics and terpenoids (Maschek
129 and Baker 2008), with the polyphenolic phlorotannins playing a primary role in maintaining
130 the cell structure of brown macroalgae, as well as fulfilling a defensive role (Arnold and
131 Targett 2003). Increased production of polyphenolics in macroalgae has been observed
132 during periods of thermal stress (Steinberg 1995), which may be an inducible defence
133 response as polyphenolics have high anti-oxidant and radical-scavenging properties (Wang
134 et al. 2009). As increased secondary metabolite production may (Johnson and Mann 1986)
135 or may not (Pansch et al. 2008) alter the palatability of kelp tissue and thereby modify
136 grazer-plant interactions, changes in production of such compounds could influence
137 ecophysiological performance of kelps at the organism and population level.

138 This study aimed to investigate the extent to which increased temperature directly and
139 indirectly influences the performance of two co-existing northeast Atlantic kelp species (Fig.
140 1), *Laminaria digitata* and *Laminaria ochroleuca*, with respect to growth rates, photosynthetic
141 efficiency, chemical defence production, and palatability to grazers. Given that in the
142 southwest UK study region *L. digitata* is found towards the trailing range edge whereas
143 marginal populations of *L. ochroleuca* represent the leading range edge (Fig. 1), we predicted
144 that high temperatures would cause a decline in the ecophysiological performance of *L.*
145 *digitata* but would be favourable for *L. ochroleuca*. We also expected production of secondary

146 metabolites to increase under stressful thermal conditions, which has the potential to alter
147 grazer-plant interactions. More generally, examining the responses of these contrasting
148 species to elevated temperature could shed new light on the underlying mechanisms driving
149 climate-mediated expansion and contraction at the range edge.

150

151 **Methods**

152 **Sample collection and field surveys**

153 To examine whether these species occupy similar environments in natural communities and
154 whether, therefore, they co-exist at their respective range edges, field surveys were
155 conducted at 2 sites within Plymouth Sound, southwest UK. At both Batten Bay and Firestone
156 Bay (Fig. 1), 10 replicate 5 x 1 m belt transects were completed in the low intertidal zone (~0.3
157 m above chart datum) during spring tide periods (March/April 2014). Transects were laid
158 parallel to the shore on suitable rocky substrata, and the abundances of *L. digitata* and *L.*
159 *ochroleuca* were recorded within each transect (only mature, distinguishable sporophytes
160 were counted; *Laminaria* recruits were not included). Transects were at least 5m apart from
161 one another. Additionally, at each site, 30 mature sporophytes of each species were randomly
162 sampled and weighed (wet weight) to estimate standing stock of each species. In order to
163 quantify the potential grazing pressure on the kelp species, the abundance of invertebrate
164 grazers associated with lamina of *L. digitata* and *L. ochroleuca* was also quantified. On low
165 intertidal platforms at 2 study sites (Batten Bay and Tinside, see Fig. 1), fine cotton mesh bags
166 were carefully placed over the laminae of randomly-selected mature sporophytes of each kelp
167 species to sample their associated macrofauna (which was subsequently identified and

168 enumerated at the laboratory). A total of 29 or 30 sporophytes of each species were collected
169 from each study site (throughout March-May 2016).

170 For the experimental work, 12 mature, medium-sized sporophytes of *L. digitata* and *L.*
171 *ochroleuca* (mean total thallus length: 109 ± 11 cm and 105 ± 16 cm, respectively) were
172 collected from the low-intertidal zone (0.2-0.4 m above chart datum) at Firestone Bay (Fig. 1)
173 in June 2015. The site is moderately exposed to waves and tidal currents, and is typical of the
174 inner shores of Plymouth Sound. There is no evidence of local anthropogenic impact, from
175 sewerage discharge or pollutants for example, and the kelp sporophytes were deemed to be
176 representative of the wider population. The site supports mixed substrata, including bedrock,
177 cobbles and boulders and patches of soft sediments. Kelp sporophytes were sampled
178 haphazardly from within a mixed kelp bed comprising *L. ochroleuca* and *L. digitata*, as well as
179 other brown macroalgae (e.g. *Saccorhiza polyschides*, *Saccharina latissima* and *Undaria*
180 *pinnatifida*, see Arnold et al. 2016 for more details).

181 In order to place our experimental temperature treatments (see below) into an ecological
182 context, we deployed temperature loggers ('Hobo' temperature pendant logger, Onset Comp.
183 Corp., Bourne) within 2 shallow subtidal habitats (at ~1 m below chart datum) in Plymouth
184 Sound, in close proximity to natural populations of *L. digitata* and *L. ochroleuca*. Temperature
185 was recorded every 30 minutes for 45 days during summer 2014 (July-September) to quantify
186 thermal maxima experienced by these populations.

187 **Experimental design**

188 An experimental tank system was used to control temperature and examine ecophysiological
189 responses to warming treatments. On return to the laboratory, the central digit of the lamina

190 of each kelp sporophyte was hole-punched with a 5mm diameter punch at 5, 10 and 15cm
191 above the stipe-meristem junction (for quantification of growth rates, see below). Each kelp
192 individual was then attached to a rock using cable ties and placed in a separate tank (121 x 38
193 x 30 cm). Replicate tanks were established (n = 4 per treatment), with 1 kelp individual in
194 each, at 3 different temperature treatments; 12°C (ambient temperature; SST data for the
195 study region showed that mean seawater temperature was ~11.5°C during the May/June
196 period), 15°C (i.e. 3°C above ambient) and 18°C (i.e. 6°C above ambient). The magnitude of
197 warming treatments was chosen to reflect temperatures observed during short-term marine
198 heatwaves in coastal ecosystems (Hobday et al. 2016). Each tank contained ~130L of seawater
199 which was circulated through header tanks and maintained at the desired temperature with
200 aquarium chillers (DC-750, Deltec, Delmenhorst) or heaters (300W DR-9300, Boyu,
201 Guangdong) as necessary. Salinity was maintained at 35 ppt with the addition of fresh water
202 as necessary; lamps (Reef Daylight T8 36W, Interpret, Dorking) specifically designed for
203 aquatic plants were used to generate a 12:12 hr light-dark regime with PAR irradiance levels
204 of ~120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (recorded with a Licor LI-250A PAR meter). Light intensity (i.e.
205 illuminance) in the tanks was monitored with duplicate 'Hobo' pendant light loggers, which
206 recorded light levels (lumens m^{-2}) every 20 minutes for 5 days. These data were compared
207 those from an identical light sensor deployed on a sub-surface pellet buoy within a shallow
208 subtidal habitat (at ~1 m below chart datum) in Plymouth Sound, which recorded light
209 intensity above the kelp canopy every 20 minutes for 5 days in May 2014. Seawater was
210 partially exchanged every 3 days to maintain water quality and nutrient levels and aquarium
211 wave-makers created turbulent water flow within the tanks. Kelp sporophytes were
212 maintained under experimental conditions for 16 days, which is representative of the
213 duration of a typical marine heat wave (Hobday et al. 2016).

214 **Response variables**

215 At the end of the experimental period, growth was quantified as the elongation rate of lamina
216 as determined from the new positions of the hole-punch marks (Parke 1948). The extension
217 distance of the lamina was then divided by the experimental duration (i.e. 16 days) to
218 calculate elongation rates (mm/day). In order to convert elongation rates to biomass
219 accumulation, the basal part of the lamina was cut transversely to form strips 50 mm in width,
220 which were individually weighed (g of fresh weight). The strip with the maximum biomass was
221 then used to calculate biomass accumulation (BA; g ind.⁻¹ d⁻¹) as $BA = xM/50d$, where x is
222 the lamina extension (mm), M is the fresh weight of the heaviest strip (g) and d is the number
223 of days between punching the hole and processing the kelp (i.e. 16 days). This method has
224 been widely used to quantify biomass accumulation in kelps (e.g. Bearham et al. 2013).
225 Measurements of lamina biomass (fresh weight) and length were also obtained. The quantum
226 efficiency of photosystem II, a measure of physiological stress, was determined using
227 standard fluorometry techniques (Murchie and Lawson 2013). F_v/F_m was measured with an
228 Aquapen AP100 fluorometer (PSI, Drasov) at the end of the experimental period. Strips of
229 basal lamina tissue (4 x 1 cm, 3 subsamples per individual) were cut, held in seawater within
230 blacked-out vessels for 15 minutes to dark adapt before being placed in a cuvette for analysis.

231 For biochemical analyses, tissue from the mid-section of the lamina (i.e. recent growth above
232 the meristematic area) was sampled from each kelp (~10 x 10 cm) and immediately frozen
233 and freeze-dried, before being ground into a fine powder. Phenols were quantified using a
234 modification of the method outlined by Van Alstyne (1988). A 100-mg sample of ground
235 freeze dried material was added to 1 ml Methanol (50 %, diluted with distilled water) in a 1.5
236 ml Eppendorf tube, vortexed and refrigerated for 24 hr. The sample was then vortexed again

237 and centrifuged, after which 0.5 ml of the supernatant was decanted into another 1.5 ml
238 Eppendorf and diluted with 0.5 ml distilled water. The sample was then vortexed and 160 μ l
239 was pipetted into a 96 well plate with 20 μ l Folin-Ciocalteu (FC) reagent (50 %, diluted with
240 distilled water). After 5 minutes 10 μ l 1.5M Na_2CO_3 was added. Absorbance was read at 765
241 nm (FLUOstar Omega microplate reader, BMG Labtech, Offenburg), with a solvent blank. Each
242 absorbance was converted to a percentage value of total dry mass using a standard curve
243 using phloroglucinol. Flavonoids were extracted to the same protocol as the phenolics, but
244 samples were not diluted prior to the assay. An 88 μ l sample solution was added to 60 μ l AlCl_3
245 (10%), and after 5 minutes 20 μ l K Acetate (3%) was added. The plate was read at 415 nm with
246 a solvent blank. The absorbance values were calculated as percentages of dry mass using
247 Quercetin as a standard. For both assays, 6 measurements were obtained for each replicate
248 kelp sample; these were treated as subsamples and averaged for each kelp.

249 To test for any variability in the palatability of algal tissue between experimental treatments,
250 the marine gastropod *Gibbula cineraria* was used as a model grazer for feeding bioassays
251 based upon techniques described by Hay et al. (1994) and Amsler et al. (2005). 'Biscuits' of
252 algal tissue, suspended in agar, were produced using a mixture of 1 g of finely ground freeze-
253 dried kelp lamina sample, 1 g of similarly prepared *Ulva* sp. (to optimise the viscosity, see Hay
254 et al. 1994) and 0.7 g agar powder (20 replicates per temperature per species, 120 in total).
255 The seaweed samples were mixed with 16 ml of deionised water, and the agar medium was
256 made up with a further 20 ml of water. The agar solution was then heated to melting point,
257 mixed with the seaweed samples and poured over glass slides (34 x 42 mm^2) covered with a
258 1 mm^2 plastic mesh. This molten mixture was then compressed between glass plates spaced
259 to give a 1 mm thick layer of nutritional medium above the mesh surface. Each 'biscuit' was

260 cut from the block of agar medium and the edges trimmed with a scalpel to make grazing
261 detection easier. Large, mature *Gibbula cineraria* individuals were collected from Firestone
262 Bay and were kept in the lab at 15°C (\pm 1°C), and deprived of food for 10 days prior to the
263 trials. To quantify total grazing individual grazers were placed in small (1 L), covered, gently
264 aerated aquaria with one biscuit (n=10 per species per temperature regime) for 48 hr, after
265 which the area grazed was quantified by counting the number of 1 mm² grid squares of
266 material consumed. To determine patterns of grazer preference, a similar protocol was
267 employed but each grazer was presented with a choice of one biscuit from each kelp species
268 in a paired choice test (n=10 per temperature treatment). Feeding trials were conducted in
269 constant temperature chambers (held at 15°C) under a 12:12 hr lighting regime.

270 **Statistical analysis**

271 For the field survey data, variability patterns in kelp density and biomass and the abundance
272 of associated grazers were examined with 2-way ANOVA, with 'site' and 'kelp' as fixed factors.
273 For the controlled experiment, differences between treatments and kelp species were
274 examined using 2-way ANOVA with 'temperature' and 'kelp' as fixed factors for all response
275 variables. Prior to analysis data were tested for homogeneity of variance (Levene's test) and
276 validity of models was confirmed by inspection of analytical residuals. Field-based data were
277 Ln(x+1) transformed prior to analysis, whereas experimental data met the assumptions of
278 ANOVA and were left untransformed for analysis. To compensate for low sample sizes in the
279 experiment all P values were based upon bootstrapped confidence intervals using 1000
280 stratified iterations per factor combination. Where significant main effects or interaction
281 terms were detected ($P < 0.05$), *post hoc* pairwise comparisons were performed (based upon
282 estimated marginal means with bootstrapped confidence intervals as above). Data from

283 feeding preference trials were analysed using paired t-tests after first confirming normality of
284 differences using Shapiro Wilks tests.

285 **Results**

286 Field surveys showed that both kelp species were present at the same sites and at similar
287 shore heights within Plymouth Sound (Fig. 2, Fig. S1). Generally, *L. digitata* formed a distinct
288 band at a greater shore height than *L. ochroleuca*, which extended into the shallow subtidal
289 zone, but at both sites these bands overlapped to form a mixed stand of *L. ochroleuca* and *L.*
290 *digitata* at ~0.0-0.2 m above chart datum. *L. digitata* was numerically dominant at the shore
291 height surveyed at both study sites (Fig. 2) but differences were non-significant (Table S1).
292 Estimates of standing biomass for each species were more comparable (Fig. 2) due to the
293 slightly greater average weight of *L. ochroleuca* sporophytes which tended to have larger
294 holdfasts and stipes, and standing biomass did not differ statistically between either sites or
295 kelp species (Table S1). The only macrofaunal grazers associated with the laminae of the
296 kelp species were 2 gastropods; the blue-rayed limpet *Patella pellucida* and the grey
297 topshell *Gibbula cineraria*. The abundance of *P. pellucida* was generally low and highly
298 variable between sporophytes; the greatest mean abundance (2.2 ± 4.0 inds. kelp⁻¹) was
299 observed on *L. digitata* at Batten Bay (Fig. 2). A significant species x site interaction was
300 observed as *L. ochroleuca* supported statistically greater abundances of *P. pellucida* than *L.*
301 *digitata* at Tinside but not Batten Bay (Table S1). The abundances of *G. cineraria* were also
302 low and variable, but were statistically greater on *L. ochroleuca* compared with *L. digitata*
303 (Fig. 2, Table S1). Temperature data from the field showed that, during summer, shallow
304 subtidal kelp populations in Plymouth Sound experience sea temperatures in excess of our
305 highest experimental treatment (Fig. 3). Indeed, observed *in situ* sea temperatures were

306 18°C or more on at least 12 days during the summer months. Average daytime light levels
307 experienced by natural populations at a field site within Plymouth Sound were comparable
308 to light levels in the experimental tanks (Fig. S3).

309 Following the 16-day manipulative experiment, ANOVA detected a significant kelp species x
310 temperature interaction term for both elongation rate and biomass accumulation (Table 1).
311 Pairwise comparisons within each level of kelp species showed that temperature had no
312 significant effect on *L. ochroleuca* whereas both growth variables were significantly reduced
313 at 18°C for *L. digitata*. Rates of elongation and biomass accumulation for *L. digitata* were ~3
314 times greater at 12°C compared with 18°C, whereas values for *L. ochroleuca* varied little
315 across the temperature range examined (Fig. 4a&b). Overall, growth rates varied markedly
316 between the 2 species, with *L. ochroleuca* exhibiting greater elongation and biomass
317 accumulation rates (Fig. 4a&b, Table 1). Differences in growth rates were not related to
318 inert-specific variability in morphology, as the biomass and length of kelp lamina did not
319 differ between the two species (Fig S3). With regards to Fv/Fm, ANOVA detected a
320 significant interaction term as variability between kelps species was not consistent with
321 temperature (Table 1; Fig. 4c). At 12°C, both kelps exhibited similar Fv/Fm values, whereas
322 at 15°C and 18°C values for *L. ochroleuca* were significantly greater than *L. digitata* (Table 1).

323 The concentrations of the defence compounds showed similar patterns in that both
324 phenolic and flavonoid production was markedly higher in *L. digitata* compared with *L.*
325 *ochroleuca* (Fig. 4d&e). Again, a significant kelp species x temperature interaction was
326 detected; temperature did not significantly affect defence compound production in *L.*
327 *ochroleuca*, whereas the concentrations of both phenolics and flavonoids in *L. digitata* was
328 significantly greater at 18°C than at 12°C (Table 1, Fig. 4d&e). The area of algal tissue grazed

329 within the 48 hr feeding trials was variable within temperature treatments (Fig. 4f). On
330 average, consumption rates for *L. digitata* tissue declined monotonically with temperature
331 with consumption of tissue held at 12°C significantly greater than for tissue held at 18°C
332 (Table 1; Fig. 4f). Consumption of *L. ochroleuca* by comparison peaked at 15°C, with
333 noticeably more *L. ochroleuca* tissue than *L. digitata* consumed at higher temperatures.
334 Results of the feeding preference trials indicated no clear preference for either species at
335 12°C and a small but significant preference for *L. ochroleuca* at 15°C and 18°C (Fig. 5).

336 **Discussion**

337 Anthropogenic climate change is driving a redistribution of marine species at a global scale
338 (Poloczanska et al. 2013; Sunday et al. 2012), which in turn is creating novel species
339 interactions and a reshuffling of communities and ecosystems (Beaugrand et al. 2013;
340 Vergés et al. 2014). However, responses to climate change have varied markedly between
341 populations, species and biogeographic regions (Poloczanska et al. 2013), as eco-
342 evolutionary context, biotic interactions and species traits have important mediating
343 influences on climate responses in both marine (Pereira et al. 2015; Sunday et al. 2015) and
344 terrestrial ecosystems (Valladares et al. 2014; Van der Putten et al. 2010). Moreover, as
345 mechanisms that underpin species range shifts will vary between the leading and trailing
346 edges, direct comparisons between functionally-similar climate change ‘winners’ and
347 ‘losers’ will improve our understanding of processes driving changing species distributions.
348 Our study has shown that realistic, observable levels of warming negatively impacted the
349 ecophysiology of a cool-water foundation species, but had no effect on its warm-water
350 congener. Moreover, the cool-water species exhibited increased production of known
351 defence compounds, which as well as serving primary functions may also serve as a

352 compensatory mechanism by reducing palatability and grazing pressure on already-stressed
353 marginal populations.

354 The Lusitanian kelp *Laminaria ochroleuca* was first recorded in the UK, in Plymouth Sound,
355 in 1946 (Parke 1948) and has seemingly proliferated at the poleward range-edge in recent
356 years, most likely in response to increasing sea temperatures (Smale et al. 2015). At our
357 field sites, which are representative of semi-sheltered shores in southwest UK, *L. ochroleuca*
358 is now a conspicuous and important component of low shore kelp canopies. Conversely, the
359 more northerly-distributed *L. digitata* is adversely affected by increasing sea temperatures
360 at its equatorward range edge and, as such, has declined in spatial extent along the coast of
361 France in recent years (Raybaud et al. 2013) and is predicted to further contract poleward in
362 the near future (Brodie et al. 2014; Smale et al. 2013). At our field sites *L. digitata* was still
363 the dominant kelp species inhabiting intertidal rocky platforms, which is typical of semi-to-
364 fully wave exposed shores in the wider Plymouth Sound region (authors' observations). It
365 remains unclear whether the abundance and extent of *L. digitata* has declined in the
366 southwest UK region, but its growth (tom Dieck 1992) and reproduction (Bartsch et al. 2013)
367 are likely to be negatively impacted by summer temperatures. Although formal tests of
368 species interactions are needed, it seems likely that the cold and warm species are
369 competing for resources (i.e. light and space) at our field sites, given that the combined
370 densities of these kelp species ranged from 5 to 8 inds m⁻², combined biomass exceeded
371 1200 g WW m⁻² and average lamina lengths were in excess of is >1 m for both species. As
372 such any changes in ecophysiological performance mediated by increased temperature may
373 alter the balance of any biotic interactions between these species.

374 In our manipulative experiment, the ecophysiology of *L. digitata* was adversely affected by
375 the high temperature treatment, the magnitude of which reflected warming anomalies
376 typical of marine heatwaves in coastal ecosystems (Hobday et al. 2016) and was frequently
377 observed within the Plymouth Sound study region. Rates of elongation and biomass
378 accumulation for *L. digitata* declined with an increase in temperature, with the highest
379 growth occurring at 12°C and the lowest at 18°C. Bolton and Lüning (1982) reported that the
380 optimum temperature for growth in *L. digitata* was 10°C, and that this thermal optimum
381 varied little between geographically isolated populations. A similar optimum temperature
382 range for growth in *L. digitata* of 10-15°C was subsequently reported by tom Dieck (1992).
383 Our 16-day exposure experiments recorded similar optimum and maximum temperatures
384 for growth. We also observed a marked decline in growth at higher temperatures, as
385 biomass accumulation and elongation rates were >3 times greater at 12°C and 15°C
386 compared with 18°C. Further work should also examine the influence of temperature on
387 erosion and production of kelp detritus, which was not considered here but may be
388 adversely affected by warming (Krumhansl et al. 2014).

389 With respect to photosynthetic activity, Roleda (2009) reported an optimum temperature
390 range between 7°C and 13°C for photosynthesis in *L. digitata*. Of the three temperature
391 treatments in this study, Fv/Fm was highest at 12°C, with a marked decline at 15°C and a
392 significant reduction at 18°C, where low Fv/Fm values (~0.6) were indicative of physiological
393 stress (Murchie and Lawson 2013). Similar responses have been reported for 3 kelp species
394 in the South Pacific, where increased temperatures decreased the efficiency of
395 photochemical processes (Cruces et al. 2012). The experiment was conducted in May/June,
396 an ecologically-important period for many *Laminaria* species which exhibit fastest annual

397 growth rates through spring and into early summer (Lüning 1979). As such, the observed
398 reduction in growth rates and photosynthetic efficiency at higher temperatures for *L.*
399 *digitata* during this period could have wider implications for benthic primary production and
400 the availability of kelp-derived material for consumers.

401 We recorded sea temperatures in excess of 18°C on multiple occasions during the summer
402 months, which will be detrimental to *L. digitata* populations inhabiting shallow subtidal
403 habitats. Previous work has shown that air temperatures during spring low tides may exceed
404 25°C on intertidal rocky reefs in the southwest UK (Moore et al. 2007), prolonged exposure
405 to which would likely result in high rates of reproductive failure and mortality (Bartsch et al.
406 2013; Bolton and Lüning 1982; tom Dieck 1992). Indeed, significant bleaching of *L. digitata*
407 sporophytes – a symptom of desiccation or temperature stress – has been observed in
408 intertidal populations during periods of high temperatures (Smale, pers obs). Given that
409 observed and predicted maximum temperatures adversely affect *L. digitata*, it is likely that
410 marginal populations at the equatorward range edge will be negatively impacted by further
411 warming, in terms of both gradual increases in mean temperature and extreme warming
412 events, perhaps leading to a poleward range contraction as has been observed in France
413 (Raybaud et al. 2013).

414 In stark contrast, none of the response variables measured for *L. ochroleuca* were
415 significantly affected by the temperature treatments. Rates of elongation and biomass
416 accumulation and Fv/Fm were greatest at the highest temperature but these differences
417 were non-significant. An optimum temperature of ~15-17°C for growth in *L. ochroleuca* has
418 been previously reported (Biskup et al. 2014; Izquierdo et al. 2002; tom Dieck 1992), and our
419 results suggest that the marginal populations found at the poleward range edge exhibit

420 similar optimum temperatures for sporophyte growth. Given that sea temperatures are sub-
421 optimum for growth and reproduction for most of the year, continued ocean warming will
422 increase the ecophysiological performance of *L. ochroleuca* populations at the poleward
423 range edge in the future. Currently, the rate of poleward spread is restricted by winter
424 thermal minima, as gametophytes cannot reproduce and sporophyte growth is arrested at
425 temperatures below 10 °C (Izquierdo et al. 2002) and intertidal populations cannot tolerate
426 low air temperatures (G. Boalch, pers com). In addition to temperature, relatively high light
427 requirements (Izquierdo et al. 2002), susceptibility to wave action (Smale and Vance 2015)
428 and competitive interactions with other kelps (Pereira et al. 2011) are acting to suppress the
429 proliferation of *L. ochroleuca* at the range edge. Should the ecophysiological performance of
430 the cold-water species *L. digitata* decline under warmer conditions, it is plausible that
431 competitive interactions between these species will be modified, consequently allowing *L.*
432 *ochroleuca* to increase its extent and abundance in the region.

433 The phenolic and flavonoid content in *L. digitata* was significantly greater in sporophytes
434 held at the highest temperature treatment. It has been shown that thermal stress can cause
435 increased production of polyphenolics in both terrestrial plants (Rivero et al. 2001) and
436 marine macroalgae (Steinberg 1995). For example, the kelp *Ecklonia radiata* exhibits
437 seasonality in polyphenolic production as higher concentrations are observed in warmer
438 compared with cooler months, which has been linked to higher ambient temperatures
439 (Steinberg 1995). Increased polyphenolic production during periods of temperature-related
440 physiological stress may be an inducible defence response, as polyphenolics have high anti-
441 oxidant and radical-scavenging properties (Wang et al. 2009). For example, Cruces et al.
442 (2012) reported an increased level of radical scavenging correlated with increased phenolic

443 levels when the kelp *Macrocystis pyrifera* was exposed to increased thermal stress and UV
444 exposure. In contrast, Simonson et al. (2015) reported no alteration in phenol production in
445 *L. digitata* across the 11-21°C temperature range, although the temperature exposures
446 lasted for 1 week rather than the >2 week exposures described here. This study suggests
447 further support for the importance of secondary metabolites, in this case polyphenols, as
448 inducible responses to thermal stress, perhaps because of their role as reactive oxygen
449 species scavengers and anti-oxidants.

450 The rate of consumption of *L. digitata* in feeding trials was lowest for the kelp tissue held at
451 the highest temperature. It is interesting to note that the decline in palatability and the shift
452 in grazing preference towards *L. ochroleuca* corresponded with the increase in phenol levels
453 in *L. digitata* observed at higher temperatures. Since secondary metabolites often fill a suite
454 of roles, such as grazer defence, anti-microbial defence, and radical scavenging, the ability
455 of *L. digitata* to increase phenolic levels during periods of thermal stress may serve a direct
456 function in primary defence as well as a secondary function in reducing palatability and
457 therefore the detrimental effects of herbivory. However, herbivory is not recognised as
458 being a major driver of kelp population structure in the study region (Smale et al. 2013), as
459 grazer densities (e.g. sea urchins, gastropods) are generally low and there have been very
460 few reports of over-grazed areas (e.g. urchin barrens). Indeed, our (albeit limited) field
461 observations showed that the densities of molluscan grazers associated with each species
462 was low and highly variable between individual kelp sporophytes (although densities of
463 *Patella pellucida* are likely to increase throughout the year, see Toth and Pavia 2002). Even
464 so, localised high densities of molluscan grazers (e.g. *Patella pellucida*) can induce kelp
465 defoliation through direct consumption and through increased dislodgement by weakening

466 tissue (Kain and Svendsen 1969; Krumhansl and Scheibling 2011; Toth and Pavia 2002). It is
467 also possible that the relatively higher palatability of *L. ochroleuca* at elevated temperatures
468 may serve to slow the range-expansion process in a fashion consistent with biotic resistance
469 to invasive species (Kimbrow et al. 2013; Parker and Hay 2005). Further work is needed to
470 understand the ecological importance of enhanced polyphenolic production under stressful
471 conditions as it may counteract, to some extent, the expected increased rate of grazer
472 consumption relative to macrophyte growth rates at higher temperatures (Gutow et al.
473 2016; O'Connor 2009).

474 There are several limitations and caveats to the current study which should be explicitly
475 examined. First, our experiments did not test for the combined effects of exposure to high
476 air temperatures and desiccation, which intertidal populations may be subjected to during
477 periods of emersion on spring low tides. Even though the low intertidal kelp populations at
478 the study sites are exposed to air for only short periods during summer (sporophytes at 0.4
479 m above chart datum were emersed for ~10 hours throughout June-September 2015),
480 consecutive acute heat stress can have considerable impacts on kelp physiology (Pereira et
481 al. 2015) and, as such, acute exposures to high air temperatures may interact with chronic
482 exposure to higher sea temperatures to impact *L. digitata* populations. Moreover, as the
483 experiment aimed to examine ecophysiological responses affected by elevated
484 temperature, we did not include a recovery period between the warming treatments and
485 the measurement of response variables. Some kelp species and life stages have the capacity
486 to recover from acute thermal stress (Ladah and Zertuche-González 2007), and further work
487 on the cumulative combined effects of chronic and acute thermal stress is needed. Second,
488 the field data presented here were used to place the experimental work into an ecological

489 context and were not collected as part of a spatially and temporally extensive survey, nor
490 did we examine the strength and direction of species interactions (i.e. competition,
491 facilitation) between these co-existing species (as well as other canopy-forming
492 macroalgae). Biotic interactions need to be considered when predicting the effects of
493 climate change on species' distribution patterns (Kordas et al. 2011; Van der Putten et al.
494 2010) and gaining a better understanding of ecological interactions between climate change
495 'winners' and 'losers' in marine ecosystems should be a priority.

496 In conclusion, our study has shed light on the likely mechanisms underpinning climate-driven
497 shifts in the extent and relative abundances of 2 habitat forming kelp species with contrasting
498 distributions. For the northern kelp *L. digitata*, sublethal effects of warming, such as
499 decreased growth and photosynthetic activity and the energetic costs associated with
500 secondary metabolite production, will manifest during thermal maxima, which will threaten
501 the persistence of marginal populations. Population responses will be primarily driven by the
502 ecophysiological effects of high temperatures, and although compensatory mechanisms may
503 reduce top-down pressure on marginal populations, this is unlikely to be of great importance
504 within the study region. Given observed and predicted sea temperatures in the northeast
505 Atlantic (Philippart et al. 2011), it is likely that *L. digitata* will continue to retract its trailing
506 range edge polewards in the near future. Conversely, the extent and relative abundance of *L.*
507 *ochroleuca* is expected to increase, at least along semi-exposed shorelines, which could
508 ultimately affect timings and rates of primary production and the overall structure of
509 intertidal communities, although further research is needed to examine likely impacts.
510 Crucially, the ecological niches of these 2 habitat-forming species do not entirely overlap
511 (Yesson et al. 2015), as *L. digitata* can tolerate greater wave exposure and extends higher up

512 the shore than *L. ochroleuca*, which is susceptible to cold damage during low winter
513 temperatures. As such, full replacement of the cold species by the warm species is unlikely to
514 occur. Better understanding of the mechanisms underpinning climate-driven range shifts of
515 kelp species is particularly important, given that they exert strong influence on the structure
516 and functioning of associated communities in temperate marine ecosystems.

517

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759

760 Table 1: Results of 2-way ANOVA tests to examine variability in (a) elongation rates, (b)
 761 biomass accumulation, (c) Fv/Fm, (d) phenolic and (e) flavonoid content and (f) palatability
 762 to grazers between kelp species and temperature (both fixed factors). Significant P values
 763 (accepted at P <0.05; confidence intervals bootstrapped) are shown in bold. Where the
 764 interaction term was significant, pairwise tests within each level of the main factor were
 765 conducted to examine the interaction further (LD = *Laminaria digitata*, LO = *Laminaria*
 766 *ochroleuca*; P values based on bootstrapped confidence intervals).

767

768 **(a) Elongation rates**

769	Source of Variation	DF	MS	F	P
770	Kelp	1	1.606	59.41	<0.001
771	Temperature	2	0.064	2.36	0.123
772	Kelp x Temperature	2	0.156	5.78	0.011
773	Residual	18	0.487	0.027	

774

775 LD 12>18, 12=15, 15>18: LO 12=15, 12<18, 15=18

776

777 **(b) Biomass accumulation**

778

779	Source of Variation	DF	MS	F	P
780	Kelp	1	0.0040	163.9	<0.001
781	Temperature	2	0.0001	0.55	0.585
782	Kelp x Temperature	2	0.0002	8.27	0.003
783	Residual	18	0.0001		

784

785 LD 12>18, 12=15, 15>18: LO 12 =15, 12=18, 15=18

786 **(c) Fv/Fm**

787

788	Source of Variation	DF	MS	F	P
789	Kelp	1	0.0147	34.069	0.002
790	Temperature	2	0.0001	0.224	0.802
791	Kelp x Temperature	2	0.0016	3.824	0.041
792	Residual	18	0.0004		

793

794 LD 12>18, 12=15, 15=18: LO 12 =15, 12=18, 15=18

795 **(d) Phenolic content**

796

797	Source of Variation	DF	MS	F	P
798	Kelp	1	1.539	45.27	<0.001
799	Temperature	2	0.138	4.04	0.039
800	Kelp x Temperature	2	0.196	5.77	0.012
801	Residual	18	0.034		

802

803 LD 12<18, 12=15, 15<18: LO 12 =15, 12=18, 15=18

804

805 **(e) Flavonoid content**

806

807	Source of Variation	DF	MS	F	P
808	Kelp	1	7.668	82.39	0.002
809	Temperature	2	0.178	1.91	0.176
810	Kelp x Temperature	2	0.437	4.70	0.023
811	Residual	18	0.093		

812

813 LD 12<18, 12<15, 15=18: LO 12 =15, 12=18, 15=18

814 **(f) Grazing rates**

815	Source of Variation	DF	MS	F	P
816	Kelp	1	30375	4.97	0.029
817	Temperature	2	43062	7.05	0.002
818	Kelp x Temperature	2	49598	8.12	<0.001
819	Residual	54	6106		

820

821 LD 12>15, 12>18, 15=18: LO 12<15, 12=18, 15>18

822

823

824 **Figure Legends**

825 **Fig. 1.** Distribution records of *Laminaria digitata* (black dots) and *Laminaria ochroleuca* (grey
826 triangles) at the scale of (a) the mid-NE Atlantic and (b) the southwest UK study region.
827 Distribution records were extracted from recognised, quality-assured biogeographic
828 information databases (specifically OBIS, GBIF and NBN). Inset map on panel (b) details the
829 Plymouth Sound study area (marked with a black box on the main map) to show the location
830 of the study sites, Firestone Bay (FSB), Batten Bay (BB) and Tinside (TS)

831 **Fig. 2.** Mean density (a) and biomass (b) of the kelps *Laminaria digitata* (black bars) and
832 *Laminaria ochroleuca* (grey bars) on low intertidal rocky reef at 2 study sites within Plymouth
833 Sound, Batten Bay and Firestone Bay. Densities of mature sporophytes were quantified in 10
834 replicate 5 x 1 m belt transects, standing biomass (g wet weight) was estimated by sampling
835 30 mature sporophytes and combining with density data. Lower plots show mean abundances
836 of the gastropod grazers (c) *Patella pellucida* and (d) *Gibbula cineraria* found on mature
837 sporophytes of *Laminaria digitata* (black bars) and *Laminaria ochroleuca* (grey bars) at 2 study
838 sites within Plymouth Sound, Batten Bay and Tinside. A total of 29 or 30 individuals of each
839 species were collected and examined from each site between March and May 2016. All values
840 are means (\pm standard error) and an asterisk indicates a significant difference between kelp
841 species (at $P < 0.05$)

842 **Fig. 3.** Observed sea temperatures at 2 shallow subtidal rocky reef habitats (~2 m depth below
843 chart datum) in the Plymouth Sound study region during summer 2014. Temperature was
844 recorded in situ every 30 minutes. Dashed line indicates the magnitude of the highest
845 temperature treatment used in the experiment (18°C)

846 **Fig. 4.** Ecophysiological responses of the kelps *Laminaria digitata* (black bars) and *Laminaria*
847 *ochroleuca* (grey bars) to temperature treatments. Bars indicate mean values (a-e: n = 4, f: n
848 = 10 ± standard deviation) for (a) elongation rates (cm day⁻¹) (b) biomass accumulation rate
849 (g wet weight day⁻¹) (c) Fv/Fm (d) phenolic content (% dry weight) (e) flavonoid content (%
850 dry weight) and (f) the rate of consumption by the gastropod grazer *Gibbula cineraria* (mm²
851 of algal tissue consumed in 48 hr). An asterisk indicates a significant difference between kelp
852 species (at P<0.05) at each temperature

853

854 **Fig. 5.** Feeding preference of *Gibbula cineraria* at the 3 different temperatures. Preference
855 expressed as amount of *L. digitata* consumed – amount of *L. ochroleuca* consumed, positive
856 values indicate a preference for *L. digitata*, negative for *L. ochroleuca*. Boxes indicate
857 interquartile range, horizontal line = medians, whiskers = range, ** = significant at p<0.01

858

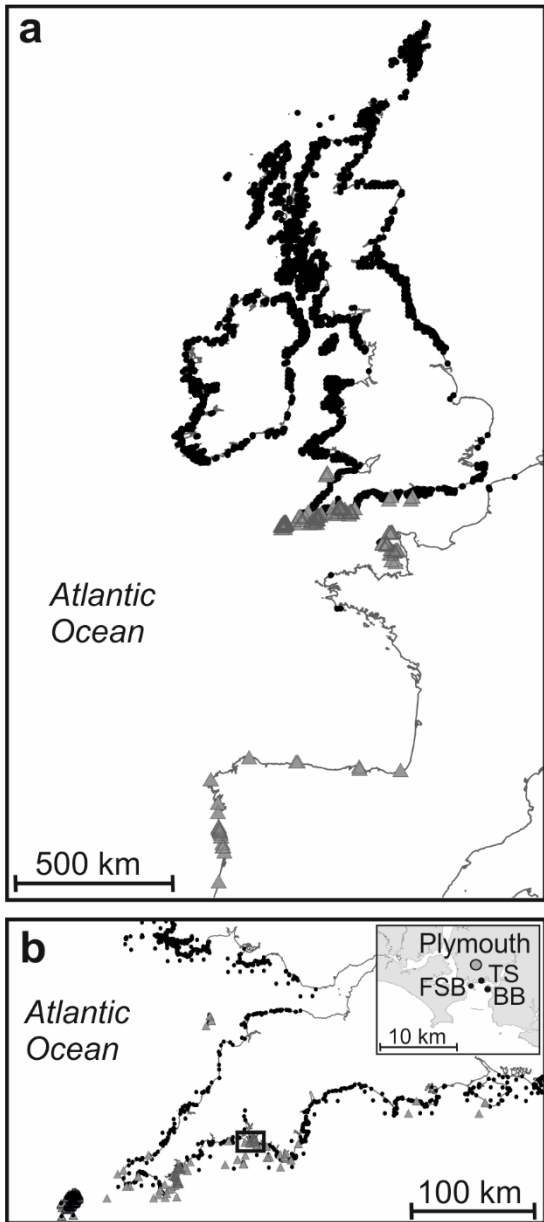


Fig. 1

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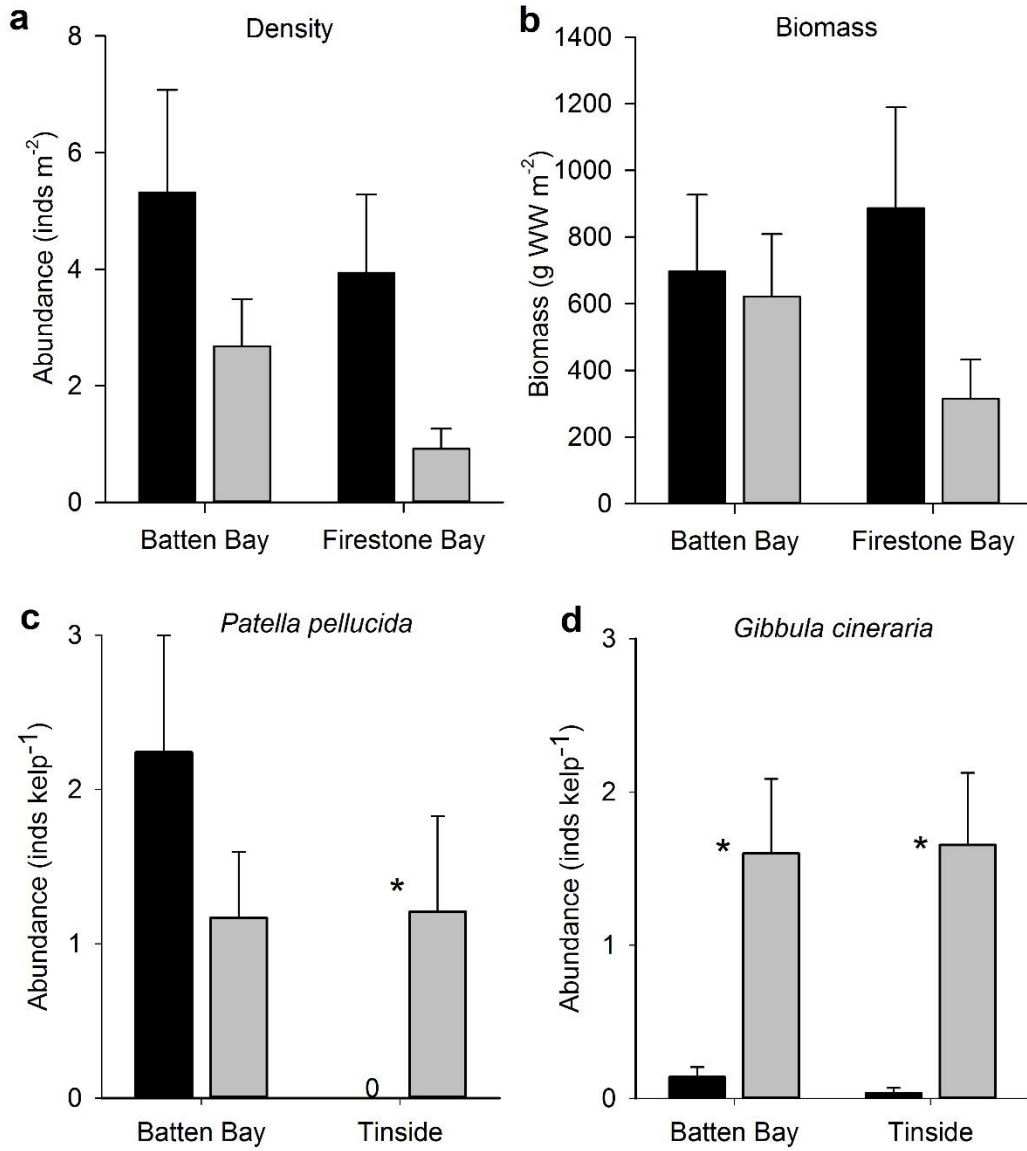


Fig. 2

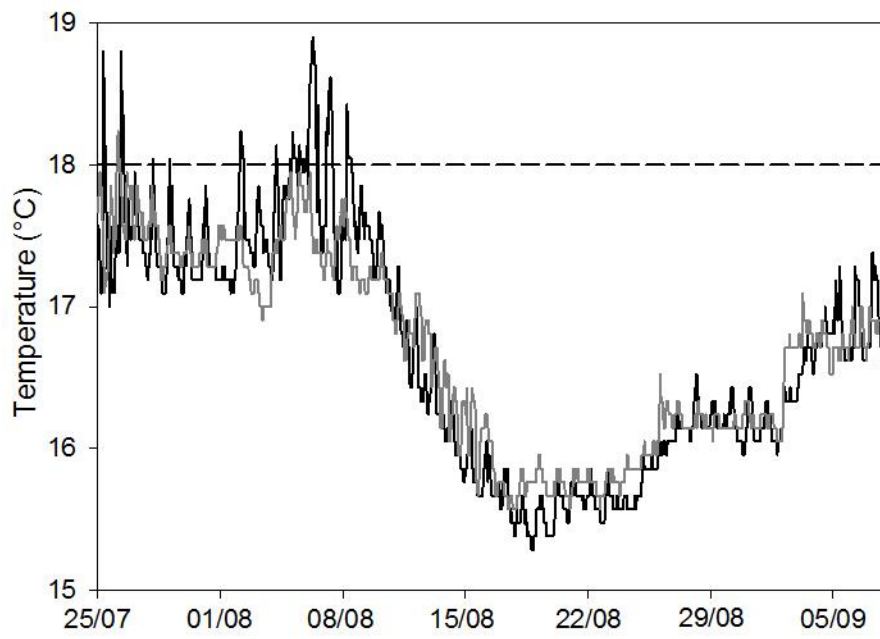


Fig. 3

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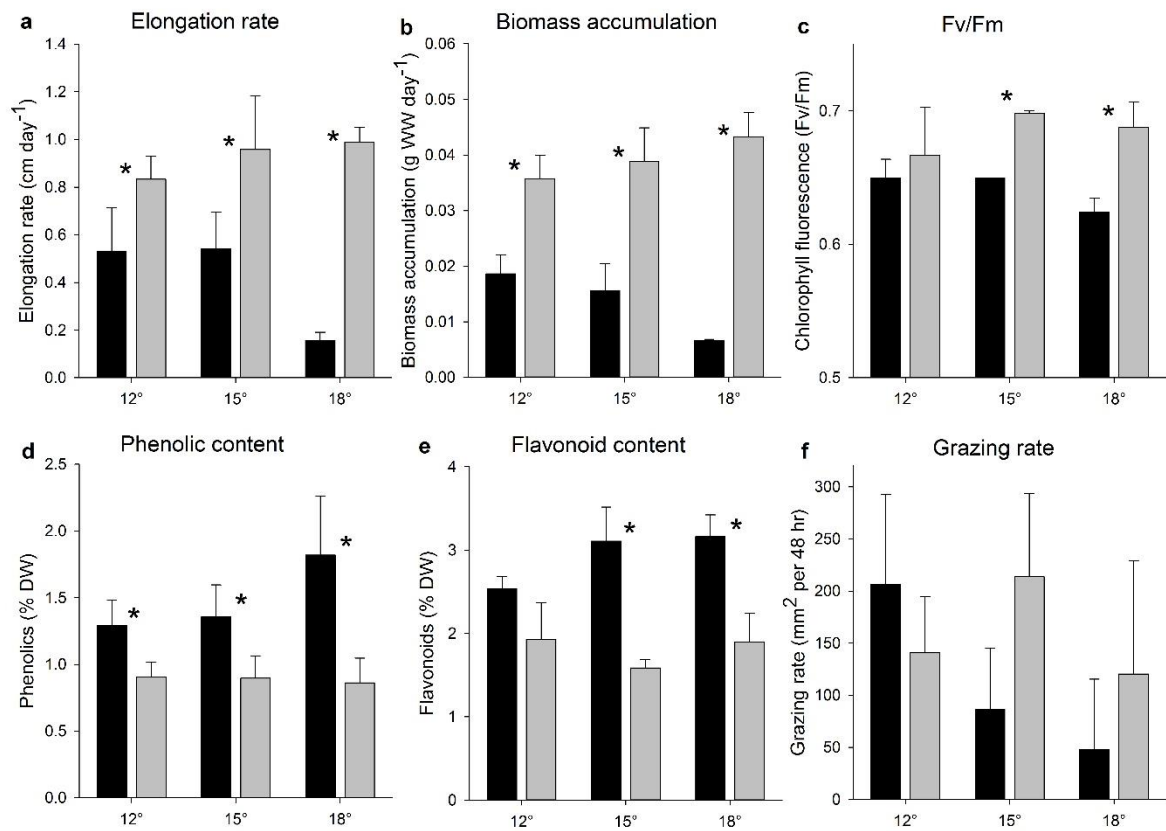


Fig. 4

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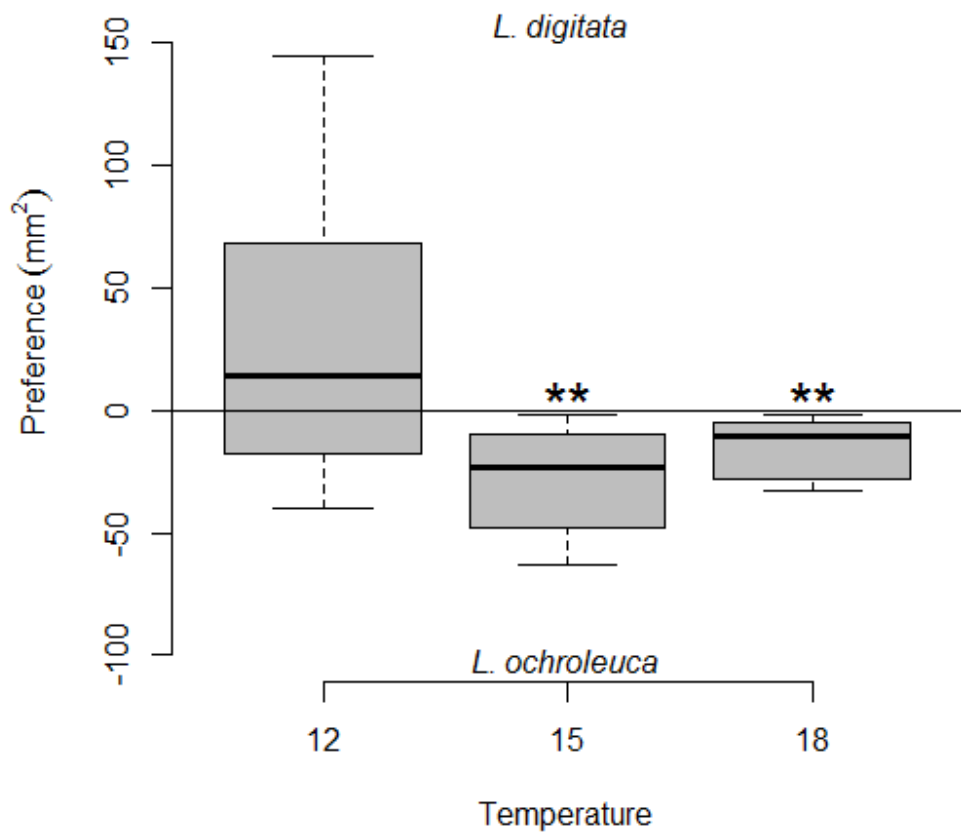


Fig. 5

863

864 Electronic supplementary material

865 ***The effects of warming on the ecophysiology of two***
866 ***co-existing kelp species with contrasting distributions***

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874

875 Table S1: Results of 2-way ANOVA tests to examine variability in (a) kelp density and (b)
 876 biomass and the abundances of associated grazers (c) *Patella pellucida* and (d) *Gibbula*
 877 *cineraria* between sites and kelp species. Significant P values (accepted at P <0.05) are
 878 shown in bold. Where the interaction term was significant, pairwise tests within each level
 879 of the site factor were conducted to examine the interaction further (BB = Batten Bay, TS =
 880 Tinside, LD = *Laminaria digitata*, LO = *Laminaria ochroleuca*). All data were Ln(x+1)
 881 transformed prior to analysis.

882 **(a) Kelp density**

883 Source of Variation	DF	MS	F	P
884 Kelp	1	5.38	3.15	0.084
885 Site	1	4.61	2.70	0.109
886 Kelp x Site	1	0.26	0.16	0.695
887 Residual	36	1.71		

889 **(b) Kelp biomass**

891 Source of Variation	DF	MS	F	P
892 Kelp	1	1.62	0.26	0.613
893 Site	1	9.95	1.59	0.215
894 Kelp x Site	1	0.01	0.01	0.988
895 Residual	36	6.26		

898 **(c) *Patella pellucida***

900 Source of Variation	DF	MS	F	P
901 Kelp	1	0.30	0.60	0.442
902 Site	1	3.44	6.83	0.010
903 Kelp x Site	1	2.16	4.30	0.040
904 Residual	114	0.50		

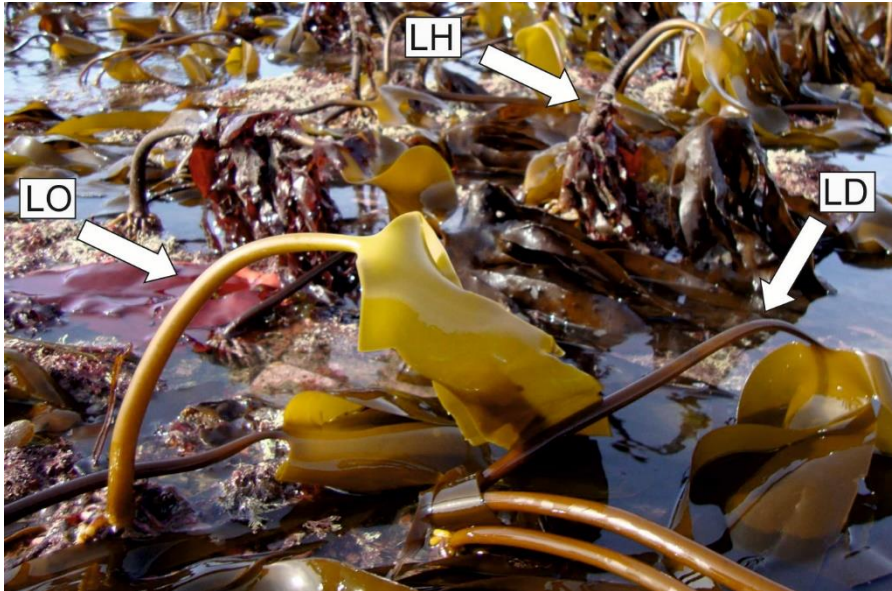
906 BB: LD=LO; TS: LD<LO

908 **(d) *Gibbula cineraria***

910 Source of Variation	DF	MS	F	P
911 Kelp	1	10.6	37.1	<0.001
912 Site	1	0.04	0.14	0.703
913 Kelp x Site	1	0.04	0.13	0.725
914 Residual	114	0.28		

916 **Fig. S1.** Low intertidal and shallow subtidal rocky reef habitats within the Plymouth Sound
917 study region support mixed kelp canopies. here showing the co-existence of the study
918 species *Laminaria ochroleuca* (LO) and *Laminaria digitata* (LD) as well as *Laminaria*
919 *hyperborea* (LH).

920



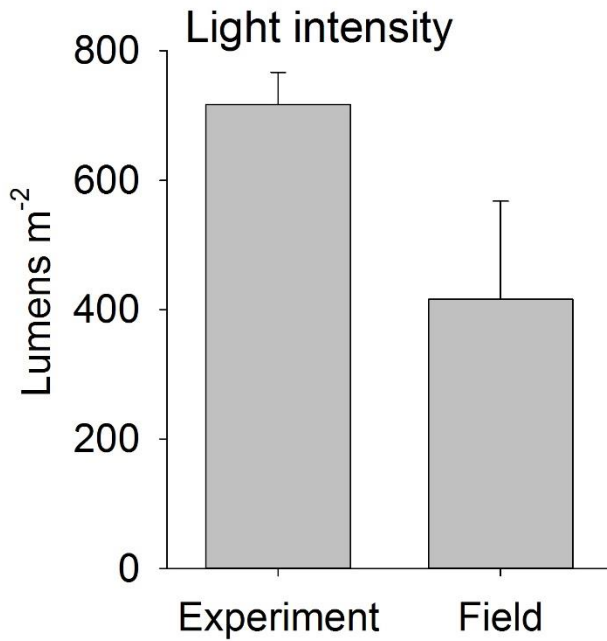
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924 **Fig. S2.** Mean daytime light intensity (12 hours of daylight between 0600 and 1800) in the
925 experimental tanks and a representative shallow subtidal (~1 m below chart datum) field site
926 within the Plymouth Sound study region. Light intensity data were captured every 20 mins
927 with 'Hobo' pendant loggers. Daily mean values are calculated from 5 consecutive days in
928 May 2014 (field) and May 2015 (tank) and shown \pm SD.

929



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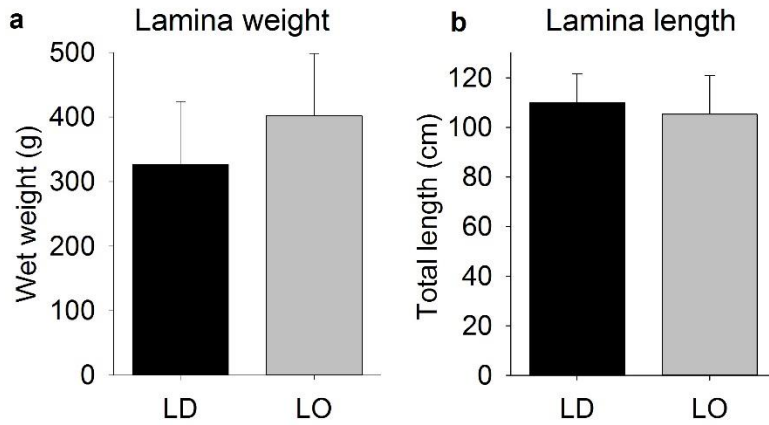
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936 Fig S3. Mean lamina biomass (wet weight) and total length for each species at the end of the
937 temperature experiment (LD = *Laminaria digitata*, LO = *Laminaria ochroleuca*). One-way
938 ANOVA indicated that both biomass ($F_{1,22} = 2.83$, $P = 0.11$) and length ($F_{1,22} = 0.66$, $P =$
939 0.43) did not differ significantly between kelp species. Mean values were generated from 12
940 replicate kelp sporophytes (shown \pm SD).

941



942

943