

Response of kelps from different latitudes to consecutive heat shock.

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Abstract

Although extensive work has focused on kelp responses to constant temperature, little is known about their response to the consecutive temperature shocks they are often exposed to in the shallow subtidal and intertidal pools. Here we characterized the responses of the two southernmost forest-forming kelp species in the Northeast Atlantic, *Laminaria ochroleuca* De

La Pylaie and *Saccorhiza polyschides* (Lightf.) Batt. to multiple cycles of thermal stress. Individuals from the upper vertical limit of the geographical distribution edges where the two species co-occur forming forests, France and Portugal, were exposed to 4 consecutive cycles of thermal shock simulating a spring tide. A 24 h cycle consisted of culture at 15 °C, plus 1 h heat shock at one of five levels (20, 22.5, 25, 27.5 or 30 °C). The maximum quantum yield (Fv/Fm) of chlorophyll fluorescence of photosystem 2 (PS2) was used to detect impaired reaction centre function, as a proxy for individual fitness costs, during recovery from heat shock. Both species showed resilience to temperatures from 20 to 25 °C. While exposure to 27.5 °C caused no inhibition to Fv/Fm of *S. polyschides*, a threshold was met above this temperature and exposure to 30 °C caused the death of all individuals. In contrast, *L. ochroleuca* from France was damaged but able to survive 30 °C shocks and individuals from Portugal showed complete resilience to this treatment. In both species, blade elongation decreased with increasing temperature, with necrosis surpassing growth at higher temperatures. Resilience to high temperature exposure may confer an advantage to *L. ochroleuca* to colonize intertidal pools on the Portuguese coast, in agreement with the observation that both species recruit in tide pools but only *L. ochroleuca* reach adulthood. Our results indicate that as summer temperatures increase with climate change, the disappearance of *S. polyschides* from intertidal pools and a decrease in the density of *L. ochroleuca* can be expected.

Keywords: *Saccorhiza polyschides*, *Laminaria ochroleuca*, chlorophyll fluorescence, heat-shock

1. Introduction

Temperature is a major factor influencing species geographical distribution, and has increased over the last decades due to anthropogenic pressure (Hampe and Petit, 2005; IPCC, 2007). Since 1980, mean sea surface temperature increased 0.2-0.3 °C per decade in southern

Europe, and 0.3-0.7 °C in the Norwegian and North seas (Lima and Wetthey, 2012). Moreover, projections indicate that warming rates may increase further in the coming decades (IPCC, 2007). These changes have the potential to cause local extinctions and poleward distribution shifts for several species (Hampe and Petit, 2005; Hiscock *et al.*, 2004; Wernberg *et al.*, 2010), as has been observed for several species of plankton (Beaugrand and Reid, 2003), gastropods (Mieszkowska *et al.*, 2006), fish (Sabatés *et al.*, 2006) and macroalgae (Nicastro *et al.*, 2013), including kelps (Díez *et al.*, 2012; Fernández 2011; Müller *et al.*, 2009; Tuya *et al.*, 2012; Voerman *et al.*, 2013). However, just as terrestrial species' distribution shifts often occur in altitude (Franco *et al.*, 2006; Kelly and Goulden, 2000), on marine shores the effects of climate change may be first observed in local species distribution on the shore, before geographical shifts are perceived (Cheung *et al.*, 2009; Pehlke and Bartsch, 2008). Species populating shallower areas such as the intertidal and upper subtidal will be the first to experience the effects of global warming, as they're more exposed to temperature extremes, UV and excessive light, desiccation and osmotic stresses, and are more exposed to storm-induced wave surge. Indeed, intertidal species have been reported to suffer shifts in their geographical distribution faster than most terrestrial species (Helmuth *et al.*, 2002). To persist as extreme environmental conditions become more frequent, populations will have to adjust to the new conditions and compensate for the elevated losses typical of populations near distributional boundaries (Guo *et al.*, 2005). As such, the resilience of recruits and early stages to repeated cycles of heat shock is likely to be a determining factor in persistence of populations in the intertidal.

Kelps are habitat structuring species. They modify the area they populate, its environment and resources, and are fundamental for the survival of several other species (Bruno *et al.*, 2003; Wernberg *et al.*, 2010). Thus, variations in kelp species composition, size and canopy density will impact ecological and oceanographic processes. Although extensive work has been done on the temperature limits of kelp species (Bolton and Lüning, 1982; Fortes and Lüning, 1990; Izquierdo *et al.*, 2002; Pang *et al.*, 2007; Pereira *et al.*, 2011; tom Dieck,

1993), nothing is yet known about their ability to cope with consecutive cycles of stress exposure associated with low tides. In this study, we aimed to recreate the conditions experienced by recruiting individuals during typical summer spring tides, where individuals may be exposed daily to repeated cycles of thermal stress during low tides, and which may be decisive for population persistence. Experiments were performed to compare the two southernmost kelp-forest species in the Northeast Atlantic: *Laminaria ochroleuca* and *Saccorhiza polyschides*. Since, as reported for several species of animals and plants, individuals from different latitudes may be locally adapted or acclimated to different conditions (Liu and Pang, 2010; Zippay *et al.*, 2010), we compared the responses of individuals from the higher and lower latitude ranges where these two species coexist: Brittany, France and Northern Portugal. The results of such experiments can provide clues as to whether temperature is likely to be a decisive factor influencing the distribution, persistence and composition of these populations in the near future.

2. Material and methods

2.1. Model species and collection

L. ochroleuca and *S. polyschides*, a perennial and an annual species, respectively, are two important Northeast Atlantic species. They both have their southernmost populations in isolated spots in the upwelling region of west Morocco and some very deep areas in the Mediterranean and Atlantic seamounts and islets, south of their coastal distributional range which ranges from the Portuguese coast to Brittany (France), and the English and Bristol Channels (Birkett *et al.*, 1998; Braud, 1974; Lüning, 1990; Norton, 1977; Titley and Neto, 2000; van den Hoek, 1982). *S. polyschides* has a wider northern distribution, found as far north as the west coast of Norway.

Sporophytes of the two species have similar upper temperature limit of around 24 °C (Birkett *et al.*, 1998; Norton, 1977). Although no records of lower temperature limits were found for either species, at their northern distribution limit average winter sea surface temperatures (SST) are 4 °C and 10 °C, for *S. polyschides* and *L. ochroleuca* respectively (Braud, 1974; Norton, 1977). Moreover, these two species have an overlapping range of optimum temperatures. *L. ochroleuca* is described as growing better between 15 and 18 °C, while *S. polyschides* seems to perform better at temperatures from 10 to 17°C (Biskup *et al.*, 2014, Izquierdo *et al.*, 2002; Norton, 1977). As such, despite their distinct life strategies, the two species have overlapping niches and are potential competitors.

This study is focused on the distributional range where the two species overlap, from northwest Iberia to the English Channel. Recruits of *L. ochroleuca* and *S. polyschides* ca. 15 cm length were collected near the northern distribution limit of *L. ochroleuca*, in Brittany, France (48°41'55.26"N 3°56'28.50"W), and in Northern Portugal (41°42'27.80"N 8°51'45.30"W), the southern distribution limit where both species are able to form forests. In Brittany, the vertical distribution of both species extends as high as the upper subtidal, being out of water only during the lowest spring tides of the year. In Northern Portugal they can be found in tidal pools and although their base is usually immersed, their blades may get exposed during spring tides. In both areas, collections were made in the upper distribution limit and were transported in cold seawater inside refrigerated boxes. In Brittany, samples were collected on the 13th of July 2011, arriving in the lab within 48h of collection. In Northern Portugal, collections were made on the 29th of March 2012, reaching the laboratory within 12 hours of collection. Experiments were done at slightly different times of the year to coincide with the recruitment peak of each area. Average SST during the month prior to collection was 14.1 °C in Brittany and 13.3 °C in Northern Portugal (Aqua MODIS SST, NASA). The same protocol and experimental conditions were used for both populations.

2.2. Experimental design

Immediately upon arrival, each individual was placed in 0.5 L individual tanks with 0.2 μm filtered seawater (FSW) and aeration at 15 °C for 5 days of acclimation, so they could recover from eventual stress caused by collection and transportation. This temperature close to the conditions during the month of collection and small sporophytes and other microscopic forms of both species grow efficiently at 15 °C (Izquierdo *et al.*, 2002; Norton, 1977; Pereira *et al.*, 2011). These conditions were also used as control during the experiment. Throughout the acclimation and experimental periods, sporophytes were exposed to a 12h day photoperiod, with a photon flux density of 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Bruhn & Gerard 1996; Izquierdo *et al.*, 2002). Seawater was changed every two days during the acclimation period. During the heat shock experiment this was done every day after exposure, to make sure individuals were not exposed to metabolites, as tidal flow would quickly wash them away.

Temperature tolerance of young sporophytes (5 replicates) was tested for a series of sequential 1 hour exposures to one of five temperature treatments; 22.5, 25, 27.5 or 30 °C. Unnaturally rapid warming was avoided by sequential transfer at each temperature, in a 30 min ramp, until the target temperature was reached. Exposure to the target temperature was for 1 hour. Afterwards, individuals were directly transferred back to the control temperature (15°C) to simulate the returning tide, and remained at the control temperature till a 24 h cycle was complete. This procedure was repeated for 4 cycles. Measurements were repeatedly performed on the same individual and each was daily exposed to the same target temperature.

2.3. Photosynthetic yield determination

Photosynthetic activity is considered to be one of the most heat sensitive cellular processes, both by damage to the oxygen-evolving complex in PS II, damage and rapid turnover of the D1 protein, and by impairment of recovery processes by reactive oxygen species. As such, *in vivo*, the efficiency of reaction center functioning (controlling electron flux for

downstream processes) represents a balance between damage and repair (Allakhverdiev *et al.*, 2008). Initial values of chlorophyll maximum quantum yield (Fv/Fm) were measured for each individual (5 replicates per treatment) under control conditions. All Fv/Fm measurements were made with a portable chlorophyll fluorometer (Junior-PAM, Walz, Germany). Individuals were dark incubated for 5 minutes, after which Fv/Fm measurements were made on two different areas, in the center of the blade of each individual's. The mean of the two values was used in downstream analyses. Because small intrinsic differences in population- and/or location-specific Fv/Fm values can obscure subsequent statistical comparisons between species and/or populations, all Fv/Fm data were normalized as a proportion of the initial values (adjusted mean=1). Initial Fv/Fm values were above 0.7 to make sure the conditions used were not stressful.

During each of 4 consecutive 24 h cycles, individuals were exposed to heat shock for one hour at the target temperature, immediately after which Fv/Fm was measured. Individuals were then returned to the control temperature (15 °C) for recovery and Fv/Fm was measured again after 24 h to assess recovery. Controls remained at 15 °C throughout each cycle, but otherwise were manipulated in same way as the other treatments. The same individuals were used throughout the experiment and were always exposed to the same target temperature.

2.4. Growth measurements

Individual growth was expressed as relative change in blade area determined from photographs taken at the beginning of the experiment, after the acclimation period, and by the end of the 4 heat shock cycles. Tissue death was considered as negative growth. The software GIMP (GNU Image Manipulation Program) 2.6.6 was used for these measurements.

2.5. Statistical analysis

PERMANOVA assesses differences in distribution, which may be caused by differences in means, in dispersion or both. PERMDISP focuses only on dispersion, complementing the PERMANOVA results. Both PERMANOVA and PERMDISP routines (Anderson *et al.*, 2008) were used to analyze the data. Maximum quantum yield was compared between species and temperatures for each location. Growth data was compared for the two species exposed at the different temperatures. No direct statistical comparisons between French and Portuguese experiments were performed because these were done in different months of different years.

3. Results

The maximum quantum yield of photosystem II (Fv/Fm) of recruits collected in Brittany (France) varied between species, temperature and time (PERMANOVA, time * species * temperature, $p < 0.0001$). At the control temperature, 15 °C, no significant difference was observed through time for either species (Fig. 1). At 22.5°C, although significant but rather small differences were observed through time, for both species, within the temperature treatment, there was no significant difference at each time point between 22.5°C and the control. Exposure to higher temperatures (25 and 27.5 °C) caused significant initial decreases in Fv/Fm in *L. ochroleuca* following stress exposure. However, recovery was complete within each 24h cycle. Thus, sublethal temperature stress in the Brittany *L. ochroleuca* population caused reversible reductions in Fv/Fm (photoprotection of PSII). However, at 30 °C, Fv/Fm declined following each consecutive stress cycle, indicating a breakdown of thermal resilience. Nevertheless, at the end of the experiment all *L. ochroleuca* individuals were still alive. A rather different strategy was seen in *S. polyschides* from Brittany, where almost no effect of temperature on Fv/Fm was observed between 15 and 27°C (Fig. 1), but where resilience collapsed after repeated exposure to 30°C. By the end of the 4th cycle no chlorophyll fluorescence could be detected for any individual, and no recovery was observed by the end of

the experiment. As the threshold temperature tolerance was approached, individual variance clearly increased relative to that under control conditions (Fig. 1).

Kelp recruits from the French populations showed no significant interspecific differences in relative growth rates. Growth rates were, however, significantly affected by temperature (PERMANOVA, temperature, $p = 0.0028$), being reduced as temperature increased to 27.5 °C for *L. ochroleuca*, and to 30 °C for both species. At higher temperatures growth was surpassed by necrosis, resulting in size decrease (Fig. 2).

Fv/Fm of recruits from Portugal varied significantly over time between species and temperatures (PERMANOVA, Time*Species*Temperature, $p < 0.0001$, Fig. 3). Data from the 15 °C treatment shows that *L. ochroleuca* and *S. polyschides* responded similarly to culture conditions, although the variations in Fv/Fm over time and between individuals were greater than observed for the Brittany populations. Significant differences between post-stress and recovery points were not observed during exposure to either 22.5, 25 °C or 27.5 °C. Exposure of *L. ochroleuca* to 30 °C revealed a clearly greater resilience in the Portuguese compared to the Brittany population (c.f. Figs 1 and 3). Although photoinhibition increased with consecutive heat-shock cycles, recovery of Fv/Fm to control levels occurred within 24 h after each exposure, indicating resilience to this level of thermal stress, as opposed to the population from Brittany. In contrast, Portuguese *S. polyschides* was even more affected by repeated exposure to 30 °C. At this temperature, no resilience was observed and cumulative damage resulted in the death of all individuals by the 3rd heat shock cycle (Fig. 3).

Relative growth of kelp recruits from Portugal varied significantly with species and temperature (PERMANOVA, species * temperature $p < 0.0001$, Fig. 2). Growth rate shows a tendency to decrease with increasing temperature for both species, but this difference was significant only for *S. polyschides*, which showed a more pronounced trend. Relative growth differed significantly between species at 30 °C only.

4. Discussion

In this study we report lethal temperature limits and sub-lethal temperature effects for recruits of the two southernmost forest-forming kelp species in the northeastern Atlantic: *L. ochroleuca* and *S. polyschides*. We also show differences between populations of each species taken from southern and northern regions where they co-occur, with evidence that *L. ochroleuca* potentially exhibits greater thermal resilience near the southern edge of its range, which might indicate some adaptation to local conditions. Such phenotypic plasticity has been reported for *S. polyschides* (Biskup *et al.*, 2014) and other kelp species, such as *Laminaria digitata* (Delebecq *et al.*, 2012), *Saccharina japonica* (Pang *et al.*, 2007) and *Ecklonia radiata* (Staeher & Wernberg, 2009; Wing *et al.*, 2007). Previous studies, based on constant growth temperature, reported that both species have a similar upper temperature limit of 23 - 25 °C (Lüning, 1990; Norton, 1977). These limits were, however, determined by exposure to a constant growth temperature, and nothing was known about the impact of the rapid and consecutive temperature rise they are often exposed to in tidal pools, a common habitat in Portugal. Because the species occupy similar areas on the shore, it was expected that their responses would be similar. While water temperature in Northern Portugal is usually about 15 °C, in tidal pools it may reach 20 °C on warmer summer days and blades may be subjected to temperatures as high as 30 °C near the surface (Engelen *et al.*, 2008). Both species showed resilience to relatively short exposures to temperatures above their reported 23 -25 °C survival limit (Lüning, 1990; Norton, 1977). Although *S. polyschides* showed no significant response when exposed to up to 27.5 °C, when subjected to 30 °C a threshold seems to have been met as individuals were significantly damaged and died. In contrast, *L. ochroleuca* appeared more resistant to extreme temperatures; individuals from Brittany were damaged by 30 °C exposure but were still alive by the end of the experiment and individuals from Northern Portugal were even more resistant, showing total resilience. Still, temperature increase caused a tendency for blade elongation to decrease and at higher temperatures necrosis surpassed growth. This also

happened for *S. polyschides* from both locations. While previous work (Biskup *et al.*, 2014) reported a higher physiological plasticity of *S. polyschides* when compared to *L. ochroleuca*, consecutive exposure to high temperatures indicates that such disparate responses are mainly a matter of strategy, most likely associated with their distinct life history. *L. ochroleuca*, as a perennial species, benefits more from photo protective mechanisms, while for the opportunistic annual, *S. polyschides*, strategies for faster growth may be selected. Although *S. polyschides* is able to recruit in tidal pools, only few individuals can be found in the summer and the main kelp species occupying such areas is *L. ochroleuca* (personal observations; Barradas *et al.*, 2011). The response of *L. ochroleuca* to short high temperature exposure suggests that its resilience provides an advantage to colonize such shallower areas. The inability of *S. polyschides* to cope with daily short exposure to 30 °C may offer an explanation as to why these are found in tidal pools in such low number and there they do not reach similar dimensions to *L. ochroleuca* nor to those attained by other *S. polyschides* in the high subtidal (personal observations).

Responses to change in environmental conditions are reported to be largely mandated by an individual's history (Lüning, 1990). Although no significant difference in Fv/Fm between sites can be asserted for *S. polyschides*, the response of *L. ochroleuca* varied between sites at 30 °C. While *L. ochroleuca* from Portugal exposed to 30 °C suffered necrosis, individuals from Brittany showed significant blade loss at temperatures above 27.5 °C, an important difference between populations. Although blade elongation is not an expression of meristematic activity, this balance between growth and necrosis is important for population persistence, particularly since an individual's spore production depends on blade length. Our finding on differences in responses to temperature between populations of *L. ochroleuca* does not match other studies on *Laminaria sp.*, where the effect of temperature on growth and survival didn't vary significantly along a latitudinal range (Bolton and Lüning, 1982; Kain, 1967). Such variation has, however, been observed for other kelp species (Liu and Pang, 2010).

Our results suggest that, in the near future, temperature will most likely not be a limiting factor for either species in the high subtidal, at least in the moderately exposed shores where they occur and where water mixing prevents strong thermal stratification at the surface. However, in tidal pools, a temperature increase might lead to higher apical blade loss and an increase in mortality rates. Moreover, climate change is accompanied by environmental changes other than temperature increase (Deysher and Dean, 1986; Han and Kain, 1996; Fortes and Lüning, 1980; Müller *et al.*, 2009) which may impair local persistence before temperature increases enough to hamper recruit survival in tidal pools. Nevertheless, our results show that temperature responses can explain subtle differences in the upper distribution of these kelp species and variation between populations along the distributional range.

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6. References

- Allakhverdiev, S.I., Kreslavski, V.D., Klimov, V.V., Los, D.A., Carpentier, R. and Mohanty, P. (2008) Heat stress: an overview of molecular responses in photosynthesis. *Photosynth res.* 98: 541-550
- Anderson, M.J., Gorley, R.N. and Clarke, K.R. (2008) PERMANOVA+ for PRIMER. Guide to software and statistical methods. PRIMER-E Ltd., Plymouth, UK. 214pp

- Barradas, A., Alberto, F., Engelen, A.H. and Serrão, E. (2011)** Fast sporophyte replacement after removal suggests bank of latent microscopic stages of *Laminaria ochroleuca* (Phaeophyceae) in tide pools in northern Portugal. *Cah Biol Mar.* **52**: 435-439
- Beaugrand, G. and Reid, P. (2003)** Long-term changes in phytoplankton, zooplankton and salmon related to climate. *Glob change biol.* **9**: 801-817
- Birkett, D.A., Maggs, C.A., Dring, M.J. and Boaden, P.J.S. (1998)** Infralitoral reef biotopes with kelp species. Vol. VII
- Bolton, J.J. and Lüning, K. (1982)** Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. *Mar Biol.* **66**: 89-94
- Braud, J.P. (1974)** Etude de quelques paramètres écologiques, biologiques et biochimiques chez une Phéophycée de côtes bretonnes. PhD thesis. *Université d'Aix Marseille II.* 99pp
- Bruno, J.F., Stachowicz, J.J. and Bertness, M.D. (2003)** Inclusion of facilitation into ecological theory. *Trends Ecol Evol.* **18 (3)**: 119-125.
- Cheung, W.W.L., Lam, V.W.Y., Sarmiento, J.L., Kearney, K., Watson, R. and Pauly, D. (2009)** Projecting global marine biodiversity impacts under climate change scenarios. *Fish and Fish.* **10(3)**: 235-251
- Deysher, L.E. and Dean, T.A. (1986)** Interactive effects of light and temperature on sporophyte production in the giant kelp *Macrocystis pyrifera*. *Mar Biol.* **93**: 17-20
- Engelen A.H., Espirito-Santo C., Simões T., Monteiro C., Serrão E.A., Pearson G.A. and Santos R.O.P. (2008). Periodicity of propagule expulsion and settlement in the competing native and invasive brown seaweeds, *Cystoseira humilis* and *Sargassum muticum* (Phaeophyta). *European Journal of Phycology*,43: 275 - 282
- Fortes, M.D. and Lüning, K. (1980)** Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgol meeresunters.* **34**: 15-29

- Franco, A.M.A., Hill, J.K., Kitschke, C., Collingham, Y.C., Roy, D.B., Fox, R., Huntley, B. and Thomas, C.D. (2006)** Impacts of climate warming and habitat loss on extinctions at species' low-latitude range boundaries. *Glob Change Biol.* **12**: 1545-1553
- Guo, Q., Taper, M., Schoenberger, M. and Brandle, J. (2005)** Spatial-temporal population dynamics across species range: from centre to margin. *Oikos.* **108**: 47-57
- Hampe, A. and Petit, R.J. (2005)** Conserving biodiversity under climate change: the rear edge matters, *Ecol Lett.* **8**: 461-467.
- Han, T. and Kain, J.M. (1996)** Effects of photon irradiance and photoperiod on young sporophytes of four species of Laminariales. *Eur J Phycol.* **31(3)**: 233-240
- Harley, C.D.G., Hughes, A.R., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F., Tomanek, L. and Williams, S.L. (2006)** The impacts of climate change in coastal marine systems. *Ecol Lett.* **9(2)**: 228-241
- Helmuth, B., Harley, C.D.G., Halpin, P.M., O'Donnell, M., Hofmann, G.E. and Blachette, C.A. (2002)** Climate change and latitudinal patterns of intertidal thermal stress. *Science.* **298**: 1015-1017
- Hiscock, K., Southward, A., Tittley, I. and Hawkins, S. (2004)** Effects on changing temperature on benthic marine life in Britain and Ireland. *Aquat Conserv.* **14 (4)**: 333-362
- IPCC (Intergovernmental Panel on Climate Change; core writing team: Pachauri, R.K. and Reisinger, A. eds) (2007)** Climate change 2007: synthesis report. Contribution of Working Groups I, II and III to the 4th assessment report of the Intergovernmental Panel on Climate Change. IPCC, Geneva. pp104
- Izquierdo, J.L., Pérez-Ruzafa, I.M. and Gallardo, T. (2002)** Effect of temperature and photon fluence rate on gametophytes and young sporophytes of *Laminaria ochroleuca* Pylaie. *Helgoland Mar Res.* **55**: 285-292.
- Kain, J.M. (1967)** Populations of *Laminaria hyperborea* at various latitudes. *Helgoland Mar Res.* **15**: 489-499

- Kelly, A.E. and Goulden, M.L. (2000)** Rapid shifts in plant distribution with recent climate change. *PNAS*. **105(33)**: 11823-11826
- Lima, F.P. and Weathey, D.S. (2012)** Three decades of high-resolution coastal sea surface temperatures reveal more than warming. *Nat Commun*. DOI: 10.1038/ncomms1713
- Ling, S.D., Johnson, C.R., Frusher, S.D. and Ridgway, K.R. (2009)** Overfishing reduces resilience in kelp beds to climate-driven catastrophic phase shift. *PNAS*. **106(52)**: 22341-22345
- Liu, F. and Pang, S.J. (2010)** Performance of growth, photochemical efficiency, and stress tolerance of young sporophytes from seven populations of *Saccharina japonica* (Phaeophyta) under short-term heat stress. *J. Appl Phycol*. **22(2)**: 221-229
- Lüning, K. (1990)** Seaweeds. Their Environment, Biogeography and Ecophysiology. *John Wiley & Sons, Inc.* 527pp
- Martínez, E.A. (1999)** Latitudinal differences in thermal tolerance among microscopic sporophytes of the kelp *Lessonia nigrescens* (Phaeophyta: Laminariales). *Pac Sci*. **53(1)**: 74-81
- Mieszkowska, N., Kendall, M.A., Hawkins, S.J., Leaper, R., Williamson, P., Hardman-Mountford, N.J. and Southward, A.J. (2006)** Changes in the range of some common rocky shore species in Britain – A response to climate change? *Hydrobiologia* **555**: 241-251
- Müller, R., Laepple, T., Bartsch, I. and Wiencke, C. (2009)** Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Bot Mar*. **52**: 617-638.
- Nicastro, K.R., Zardi, G.I., Teixeira, S., Neiva, J., Serrão, E.A. and Pearson G.A. (2013)** Shift happens: trailing edge contraction associated with recent warming trends threatens a distinct genetic lineage in the marine macroalgae *Fucus vesiculosus*. *BMC Biol*. **11**:6
- Norton, P.A. (1977)** Experiments on the factors influencing the geographical distribution of *Saccorhiza polyschides* and *Saccorhiza dermatodea*. *New Phytol*. **78**: 625-635
- Pang, S.J., Jin, Z.H., Sun, J.Z. and Gao, S.Q. (2007)** Temperature tolerance of young sporophytes from two populations of *Laminaria japonica* revealed by chlorophyll fluorescence

measurements and short-term growth and survival performance in tank culture. *Aquaculture*. **262**: 493-503

Pehlke, C. and Bartsch, I. (2008) Changes in the depth distribution and biomass of sublittoral seaweeds in Helgoland (North Sea) between 1970 and 2005. *Clim Res*. **37**: 135-147

Pereira, T.R., Engelen, A.H., Pearson, G.A., Serrão, E.A., Destombe, C. and Valero, M. (2011) Temperature effects on the microscopic haploid stage development of *Laminaria ochroleuca* and *Saccorhiza polyschides*, kelps with contrasting life histories. *Cah Biol Mar*. **52**: 395-403

Sabatés, A., Martín, P., Lloret, J. and Raya, V. (2006) Sea warming and fish distribution: the case of the small pelagic fish, *Sardinella aurita*, in the western Mediterranean. *Glob Change Biol*. **12**: 2209-2219

Tittley, I. and Neto, A.I. (2000) A provisional classification of algal-characterized rocky shore biotopes in the Azores. *Hydrobiologia*. **440**: 19-25

tom Dieck, I. (1993) Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. *Mar Ecol Prog Ser*. **100**: 253-264

Van den Hoek, C. (1982) The distribution of benthic marine algae in relation to the temperature regulation of their life histories. *Biol J Linn Soc*. **18**: 81-144

Wernberg, T., Thomsen, M.S., Tuya, F., Kendrick, G.A., Staehr, P.A. and Toohy, B.D. (2010) Decreasing resilience of kelp beds along a latitudinal temperature gradient: potential implications for a warmer future. *Ecol Lett*. **13**: 685-694.

Zippay, M.L. and Hofmann, G.E. (2010) Physiological tolerances across latitudes: thermal sensitivity of larval marine snails (*Nucella spp*). *Mar Biol*. **157(4)**: 707-714

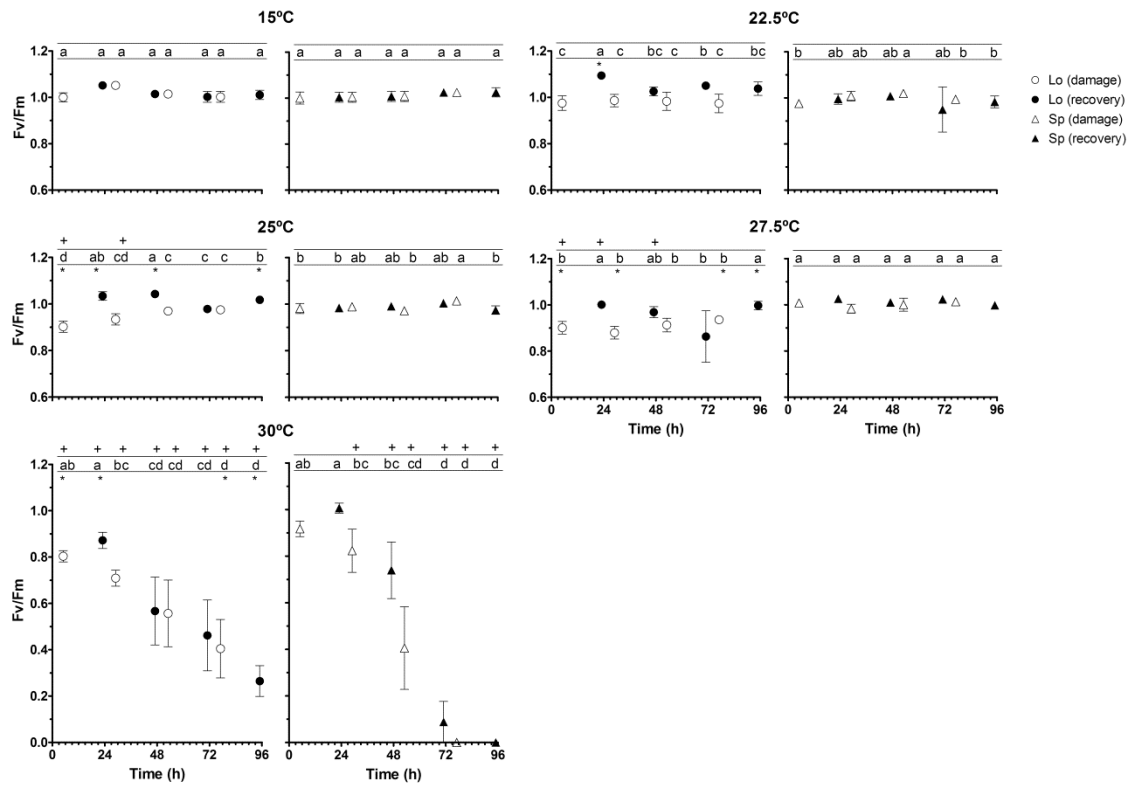


Figure 1

Fig.1: Fv/Fm variations of *Laminaria ochroleuca* (left, circles) and *Saccorhiza polyschides* (right, triangles) from Northern Brittany, France, in response to exposure to repeated 1 hour temperature elevation. Error bars represent standard error (n=5). Open symbols indicate measurements post-stress. Closed symbols refer to measurements after the recovery period. + indicates a significant difference from the control (15°C). * indicates a significant difference between the two species. Different letters were attributed, per species and temperature, to significantly different means.

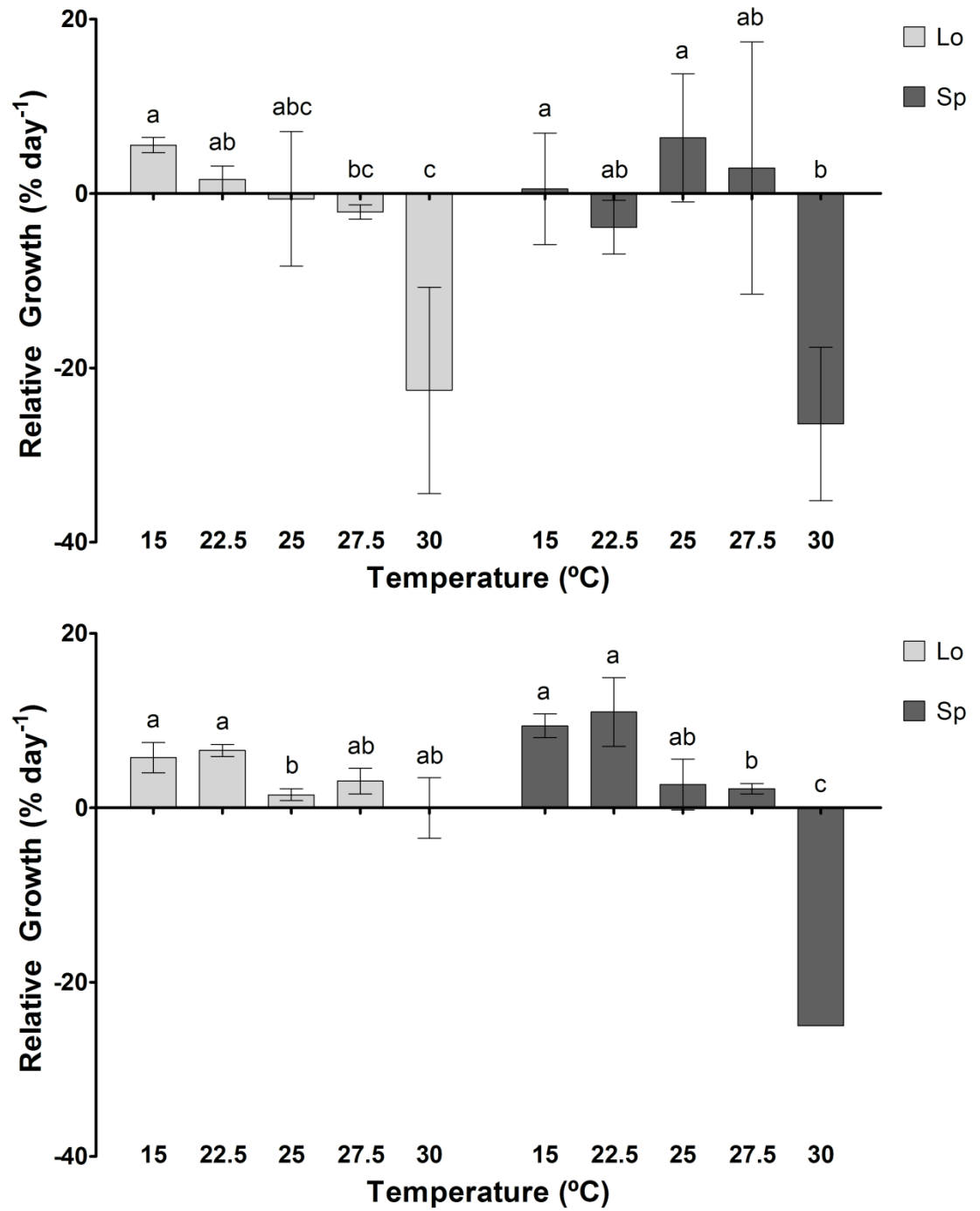


Figure 2

Fig. 2: Mean relative blade area growth rate ($n = 5$) of *L. ochroleuca* (Lo) and *S. polyschides* (Sp) from Brittany, France (top) and Northern Portugal (bottom), cultivated at 15 °C with daily recurrent exposures of one hour to 15, 22.5, 25, 27.5 and 30 °C. Error bars represent standard error. Different letters were assigned, per species, to points with significantly different means ($p < 0.05$). No significant difference was observed between species at identical temperature treatments.

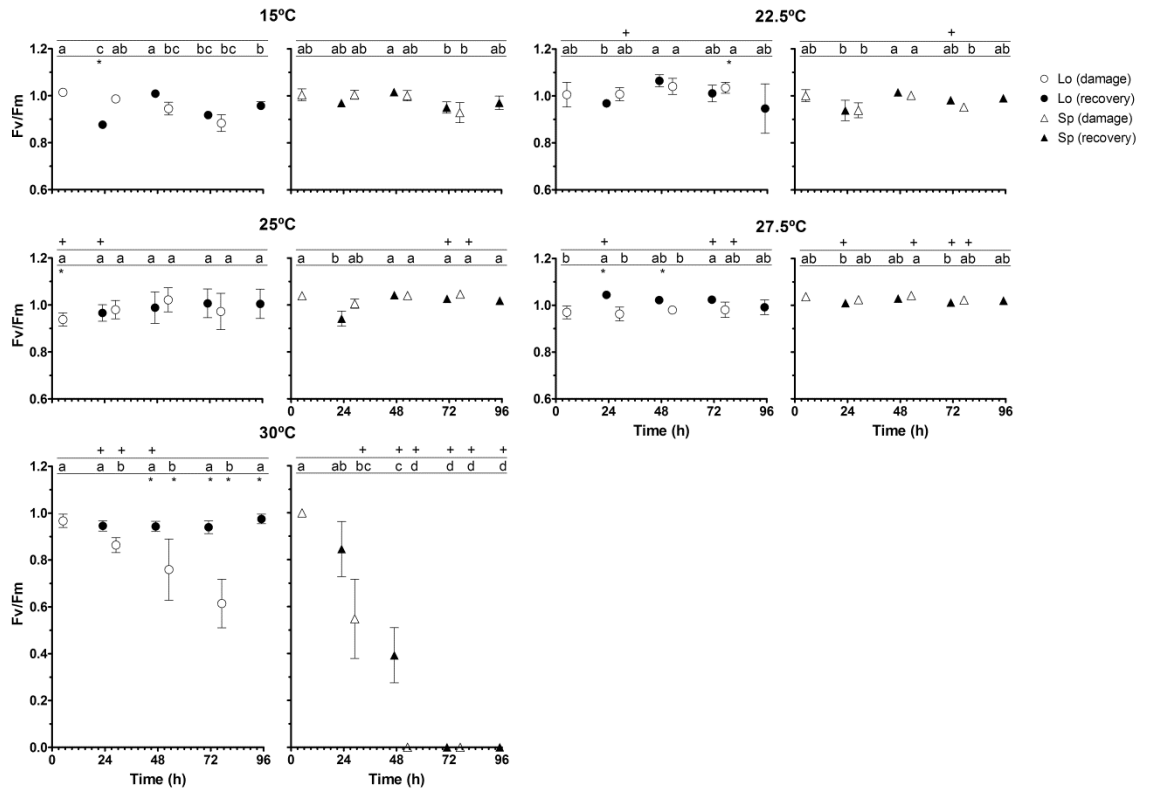


Figure 3

Fig.3: Fv/Fm variation with repeated exposure to high temperature in *Laminaria ochroleuca* (left, circles) and *Saccorhiza polyschides* (right, triangles) from Northern Portugal. Error bars represent standard error (n=5). Open symbols indicate measurements after the heat shock. Closed symbols refer to measurements after the recovery period. + indicates a significant difference from the control (15 °C). * indicates a significant difference between the two species. Different letters were attributed, per species, within each temperature, to significantly different points.