



Modulation of physiological performance by temperature and salinity in the sugar kelp *Saccharina latissima*

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SUMMARY

The sugar kelp *Saccharina latissima* experiences a wide range of environmental conditions along its geographical and vertical distribution range. Temperature and salinity are two critical drivers influencing growth, photosynthesis and biochemical composition. Moreover, interactive effects might modify the results described for single effects. In shallow water coastal systems, exposure to rising temperatures and low salinity are expected as consequence of global warming, increased precipitation and coastal run-off. To understand the acclimation mechanisms of *S. latissima* to changes in temperature and salinity and their interactions, we performed a mechanistic laboratory experiment in which juvenile sporophytes from Brittany, France were exposed to a combination of three temperatures (0, 8 and 15°C) and two salinity levels (20 and 30 psu (practical salinity units)). After a temperature acclimation of 7 days, sporophytes were exposed to low salinity (20 psu) for a period of 11 days. Growth, and maximal quantum yield of photosystem II (F_v/F_m), pigments, mannitol content and C:N ratio were measured over time. We report for the first time in *S. latissima* a fivefold increase in the osmolyte mannitol in response to low temperature (0°C) compared to 8 and 15°C that may have ecological and economic implications. Low temperatures significantly affected all parameters, mostly in a negative way. Chlorophyll *a*, the accessory pigment pool, growth and F_v/F_m were significantly lower at 0°C, while the de-epoxidation state of the xanthophyll cycle was increased at both 0 and 8°C compared to 15°C. Mannitol content and growth decreased with decreased salinity; in contrast, pigment content and F_v/F_m were to a large extent irresponsive to salinity. In comparison to *S. latissima* originating from an Arctic population, despite some reported differences, this study reveals a remarkably similar impact of temperature and salinity variation, reflecting the large degree of adaptability in this species.

Key words: brown algae, global warming, growth, Laminariales, mannitol, photosynthesis, pigments, seaweed.

ecosystems (Dayton 1985; Bartsch *et al.* 2008). *Saccharina latissima* is a common kelp species in temperate to polar rocky shores in the northern hemisphere. Its distribution in Europe ranges from the Arctic (Spitsbergen, Svalbard) to the north of Portugal (Araújo *et al.* 2016; Neiva *et al.* 2018). Recent reports of local extinction and range shifts (e.g. Moy & Christie 2012; Araújo *et al.* 2016) as result of environmental change might compromise the survival of the species and its associated ecosystem functions (Harley *et al.* 2012). The biochemical composition of seaweeds is modulated by environmental factors, such as temperature, salinity, light and nutrients (Stengel *et al.* 2011). Therefore, several studies provide chemical composition profiles of field-collected kelps by season and geographical location (e.g. Fernandes *et al.* 2016; Schmid *et al.* 2017). However, to elucidate how abiotic factors, singly or in interaction, drive biochemical composition laboratory experiments with cultivated material are necessary to exclude confounding effects. In addition, multifactorial designs allow the exploration of interactive effects in a way that may better allow relation to field conditions than unifactorial investigations without the associated confounding effects (Davison 1987).

Temperature is a major factor driving distributional ranges both at global and local scale by modulating survival, growth and reproduction of macroalgae (van den Hoek 1982; Lüning 1990; Lima *et al.* 2007; Harley *et al.* 2012). Temperature influences photosynthesis of macroalgae by regulating photosynthetic efficiency and pigment concentrations (Davison 1987; Andersen *et al.* 2013). Furthermore, salinity variation significantly influences the physiology and biochemistry of seaweeds which affects growth and survival (e.g. Gordillo *et al.* 2002; Spurkland & Iken 2011). In Phaeophyceae, besides its role as storage compound, mannitol also has a function in osmotic adjustment and therefore concentration changes may follow variation in the salinity regime (Iwamoto & Shiraiwa 2005; Gylle *et al.* 2009).

INTRODUCTION

Kelps (order Laminariales, class Phaeophyceae) are important primary producers and ecosystem engineers in coastal

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Subtidal kelps, such as *S. latissima*, are locally and intermittently subjected to hyposalinity driven by precipitation events, tidal ranges and freshwater input that often occur in short-time scales – a few hours to a day. In the case of freshwater input from rivers or glaciers, exposure to low salinity might last longer (for several days) depending on the geomorphology of the site (Lüning 1990; Borum *et al.* 2002; Mortensen 2017). Moreover, *S. latissima* is known to inhabit brackish waters such as the Baltic Sea (Nielsen *et al.* 2016) and the intertidal in British Columbia, Canada which grants long-term exposure to hyposalinity (Druehl & Hsiao 1977). Hence, *S. latissima* has been reported to survive salinities of 10 psu (practical salinity units) and to grow above 13 psu (Karsten 2007; Spurkland & Iken 2011). Nevertheless, the interaction of temperature and salinity on macroalgal performance has been rarely investigated. Understanding acclimation strategies to both temperature and salinity changes will support conservation practices and shed light on ecological consequences of local environmental changes.

Additionally, interest in the cultivation of *S. latissima* in Europe and North America is rising given the emergent food and industrial applications (e.g. Sanderson *et al.* 2012; Boderskov *et al.* 2016; Fernand *et al.* 2017). Namely, certain kelp pigments, such as carotenoids, and mannitol are relevant for human health (Iwamoto & Shiraiwa 2005; Wells *et al.* 2017). *S. latissima* has been traditionally harvested and it is recently also commercially cultivated in Brittany, France (Mesnildrey *et al.* 2012; Bernard *et al.* 2019). In Roscoff, Brittany, *S. latissima* is exposed to high tidal coefficients (Gévaert *et al.* 2003) and low tides might lead to exposure to low salinity during rain and hyperosmotic stress when desiccated (Lüning 1990). Therefore, understanding how chemical profiles of *S. latissima* react to changes in abiotic factors can support site selection for commercial purposes (Hafting *et al.* 2015).

To investigate acclimation mechanisms to temperature and salinity and their interaction, the physiological and biochemical responses of juvenile sporophytes of the sugar kelp *S. latissima* to variations in both abiotic factors were investigated under controlled laboratory conditions within 18 days. Thereby, this study further explores the physiological capacity of *S. latissima*, which may help to interpret its wide response plasticity along the distributional gradient. Data reported here for *S. latissima* from Roscoff, Brittany is compared to results from a study with Arctic material from Spitsbergen which took place under the same experimental conditions (Li *et al.* 2020). Both studies were performed under one common hypothesis: We assumed that material from both locations represent locally adapted ecotypes (Kawecki & Ebert 2004), and thereby we expected that our Brittany material would grow and perform better at 15°C and saline conditions than the Arctic material. In turn, we expected the Arctic material to perform better at low temperature and low salinity (0°C, 20 psu) than algae from Brittany.

MATERIALS AND METHODS

Algal material and experimental design

Young sporophytes of *Saccharina latissima* (L.) C.E. Lane, C. Mayes, Druehl, et G. W. Saunders were raised from stock

cultures of uniparental male and female gametophytes (AWI culture numbers 3425, 3426 isolated 2013 from Roscoff, 48° 43' 39" N, 3° 59' 13.2" W; Brittany, France) as described by Heinrich *et al.* (2012). Sporophytes were cultivated in 5 L glass beakers at 8°C under a photon fluence rate of 20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ of photosynthetically active radiation (PAR) (Mitras Lightbar Daylight 150, GHL, Germany) in a 18:6 h – light:dark (LD) photoperiod in an environmentally controlled room. Algae were cultivated for 3 months in sterile seawater enriched with Provasoli (Starr & Zeikus 1993; modifications: HEPES-buffer instead of TRIS, double concentration of $\text{Na}_2\text{glycerophosphate}$, iodine enrichment after Tatewaki (1966)) with a salinity of approximately 30 psu until they reached a length of 5–7 cm with an average fresh weight of 0.58 g. At the start of the experiment, sporophytes were directly exposed to the temperatures 0, 8 and 15°C for 7 days ($n = 5$). This was regarded as the temperature acclimation phase. Temperature levels were chosen to include a control temperature (8°C) that falls in the middle of the temperature range suitable for *S. latissima* growth (Bolton & Lüning 1982) and a higher (15°C) and lower value (0°C) that are locally experienced by *S. latissima* throughout the distributional range (15°C in Brittany (SOMLIT 2017) fr/fr/, 0°C in the Arctic (COSYNA Data 2017)). After 7 days, sporophytes were directly exposed to a low salinity treatment (20 psu) while the control was kept at the original salinity (30 psu) by each temperature for 11 days resulting in a total of 18 experimental days in 5 L beakers with 12 sporophytes each ($n = 5$) (see Appendix S1 in the Supporting Information for experimental design and physiological and biochemical parameters measured). The Provasoli solution was added to the low-salinity treatment after the latter was prepared by mixing deionized water with seawater. Physiological and biochemical parameters were measured in five replicates unless practical limitations during experiment and technical analysis reduced this number.

Physiological parameters

During the experimental phase (18 days) fresh weight ($n = 3$) of spot-labeled sporophytes was measured twice a week by an analytical digital balance Sartorius LA310S (Göttingen, Germany) after blotting dry. Biomass increase was calculated as percentage of initial fresh weight (day 0) to account for natural variability among replicates.

Maximal quantum yield of photosystem II (F_v/F_m , $n = 5$) of a randomly sampled sporophyte was measured twice a week with an Imaging PAM maxi-version (Pulse Amplitude Fluorometer; Walz, Effeltrich, Germany) after 10 min dark acclimation in seawater. Initial values of F_v/F_m (~0.5) were slightly lower than the value considered healthy for brown algae (~0.7) (Dring *et al.* 1996; Hanelt 1998) most likely due to the use of the Imaging PAM that often records lower values than other PAM instruments (Nielsen & Nielsen 2008).

Biochemical parameters

One sporophyte per replicate and per sampling day (see Appendix S1 in the Supporting Information) was randomly sampled for biochemical analyses (pigments, mannitol and carbon to nitrogen ratio (C:N)). Samples were frozen in liquid nitrogen,

stored at -80°C and then lyophilized with a freeze-dryer alpha 1–4 LD plus (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) for 24 h at 1 mbar and -55°C .

Mannitol content was measured on days 8, 11 and 18 ($n = 3$). For the extraction, lyophilized and homogenized three aliquots of 8–10 mg sample were incubated with 1 mL aqueous ethanol (70%, v/v) for 3–4 h in a water bath at 70°C . The mannitol concentration was determined after Diehl *et al.* (2020), using the method described in Karsten *et al.* (1991a). D(–)-mannitol standards ($\text{C}_6\text{H}_{14}\text{O}_6$, Roth) of 1, 6 and 10 mM were used for calibration. Mannitol content was calculated in mg g^{-1} dry weight (DW).

C:N ratios were analyzed on samples taken on days 8, 11 and 18 ($n = 4$) following Graiff *et al.* (2015). 2–3 mg of lyophilized and ground samples were weighed and packed into tin cartridges ($6 \times 6 \times 12$ mm) and combusted at 950°C . The content of C and N were quantified automatically in an elemental analyzer (Vario EL III, Elementar, Langensfeld, Germany). The standard used was acetanilide ($\text{C}_8\text{H}_9\text{NO}$). Total C and total N content were calculated in mg g^{-1} dry weight (DW). The C:N ratios were calculated based on these results.

Pigment content was determined on samples taken on days 8 and 18 ($n = 5$) using a high performance liquid chromatography (HPLC) following the protocol by Koch *et al.* (2016), with minor changes – no guard cartridge was used here and the samples were filtered through a $0.2 \mu\text{m}$ filter. The accessory pigment pool (Acc.) was calculated by adding chlorophyll *c2* and fucoxanthin. The xanthophyll cycle pigment pool (VAZ) is the sum of the pigments violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z). The de-epoxidation state (DPS) was calculated based on the formula $\text{DPS} = \frac{Z+0.5A}{V+A+Z}$ as described in Colombo-Pallotta *et al.* (2006).

Statistical analysis

We performed a Grubb's test to detect outliers (Grubbs 1969) through the webpage GraphPad (<https://www.graphpad.com/quickcalcs/grubbs1/>; $P < 0.05$). For subsequent analysis one outlier found in the values of VAZ and another one in the DPS values were excluded. Statistical analyses were performed with the software IBM SPSS statistics version 25. The normality assumption was tested with the Shapiro–Wilk test and the homogeneity of variances assumption with the Levene's test ($P > 0.05$). A repeated measures ANOVA was applied to fresh weight with between-subjects factors temperature and salinity and within-subjects factor time. To test the effect of temperature on F_v/F_m during the temperature acclimation phase, a non-parametric test was applied to day 4 and a one-way ANOVA to day 7. When data complied with the assumptions, separate two-way ANOVAs with temperature and salinity as fixed factors were applied to pigment content on days 8 and 18, to F_v/F_m on days 8, 11, 14 and 18, to mannitol and C:N ratio on days 8, 11 and 18. When data failed to comply with the normality and homogeneity of variances assumptions, non-parametric tests were applied – independent samples Kruskal–Wallis test for the effect of temperature and Mann–Whitney *U* test for the effect of salinity. Following the statistical tests above, Post-Hoc multiple comparisons per factor

were performed with Bonferroni corrections to identify the significant differences among treatments.

RESULTS

Physiological parameters

Fresh weight was significantly affected by the interaction of time and temperature and by the main effect of time. After 7 days of temperature acclimation, fresh weight (as percentage of initial) was 27% higher at 8°C than at 0°C (non-significant after Bonferroni adjustment for multiple comparisons, $P = 0.062$) but not different from 15°C . Fresh weight was overall 19% significantly higher on day 7 than on day 4 (Fig. 1, Appendix S2 – Table 1 in the Supporting Information).

During the subsequent salinity \times temperature treatment, there was a significant interaction between time and temperature, between time and salinity but not between temperature and salinity on fresh weight. Main effects of temperature and salinity were significant (Appendix S2 – Table 2 in the Supporting Information). Overall fresh weight was significantly higher at 30 psu than at 20 psu on days 11 (24%) and 18 (36%) and significantly lower at 0°C than at 8°C on days 14 (145%) and 18 (145%), while no significant differences were found between 15°C and 0°C or 8°C . Fresh weight significantly increased from day 8 to days 11, 14 and 18 at 0 and 15°C and significantly increased in each measuring day at 8°C (Fig. 1).

After 7 days of temperature acclimation, maximal quantum yield of photosystem II (F_v/F_m)

F_v/F_m was significantly higher at 15°C than at 8°C (0.3%) and 4.5% higher at 8°C than at 0°C ($15^{\circ}\text{C} > 8^{\circ}\text{C} > 0^{\circ}\text{C}$). On day 11, F_v/F_m was significantly affected by the interaction of temperature and salinity. F_v/F_m was significantly higher at 30 psu than 20 psu at 0°C , but not at 8 and 15°C . At the end of the salinity treatment (day 18), the interaction of temperature and salinity could not be tested due to the application of non-parametric tests. Quantum yield at 15°C again was significantly higher than at 8°C (9.2%); not significant differences were observed between 8 or 15°C and 0°C . Salinity did not significantly affect F_v/F_m on day 18. (Fig. 2, Appendix S2 – Table 3 in the Supporting Information).

Biochemical parameters

At the end of the experiment, on day 18, no significant interaction between temperature and salinity and no effect of salinity on pigment content was observed. Acc. and DPS were only significantly affected by temperature, but not Chl *a* and VAZ. While DPS was significantly lower at 15°C than at 0 and 8°C (219%), Acc. content was significantly higher at 15°C than at 0°C (185%) and there was no significant difference to 8°C (Fig. 3 – Appendix S2 – Table 4 in the Supporting Information). On day 8 (24h of salinity exposure), VAZ was affected by the interaction of temperature and salinity while Chl *a* (chlorophyll *a*), Acc. and DPS were all significantly affected by temperature alone but not by salinity (Appendix S3 in the Supporting Information).

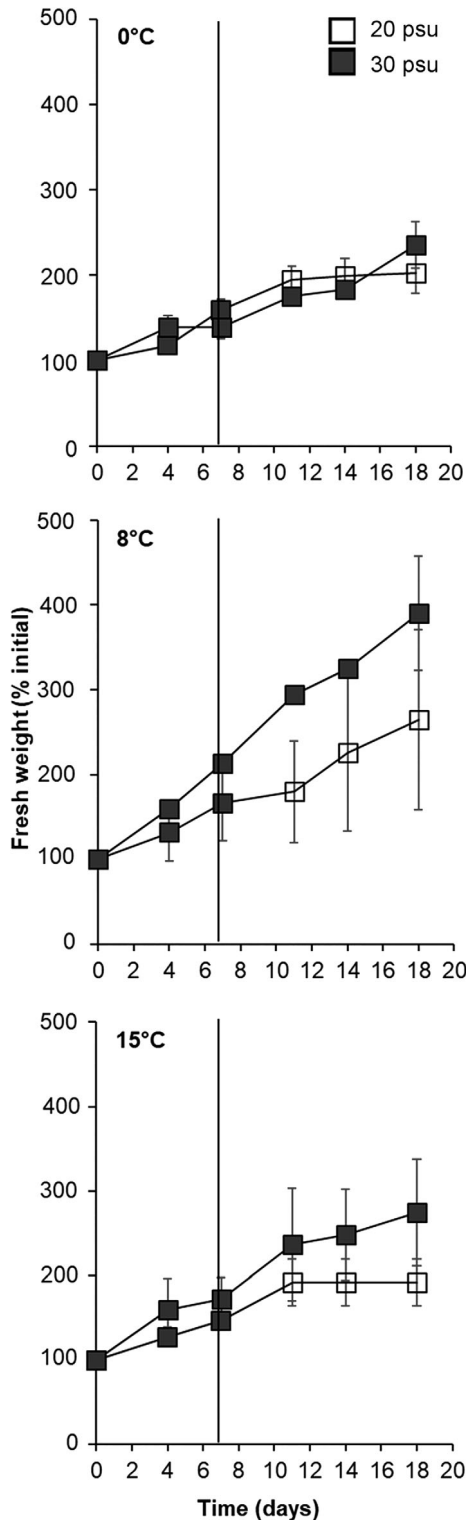


Fig 1. Fresh weight of *Saccharina latissima* from Brittany at day 0, 4, 7, 11, 14 and 18 at 0, 8 and 15°C (% of initial weights; mean \pm SD; $n = 3$). Grey boxes mark 30 psu, white boxes 20 psu. On the left, temperature acclimation phase – 7 days at 0, 8, 15°C and control salinity 30 psu; and on the right, hyposalinity exposure phase – sporophytes were exposed to low (20 psu) and control (30 psu) salinity at 0, 8, 15°C for 11 days.

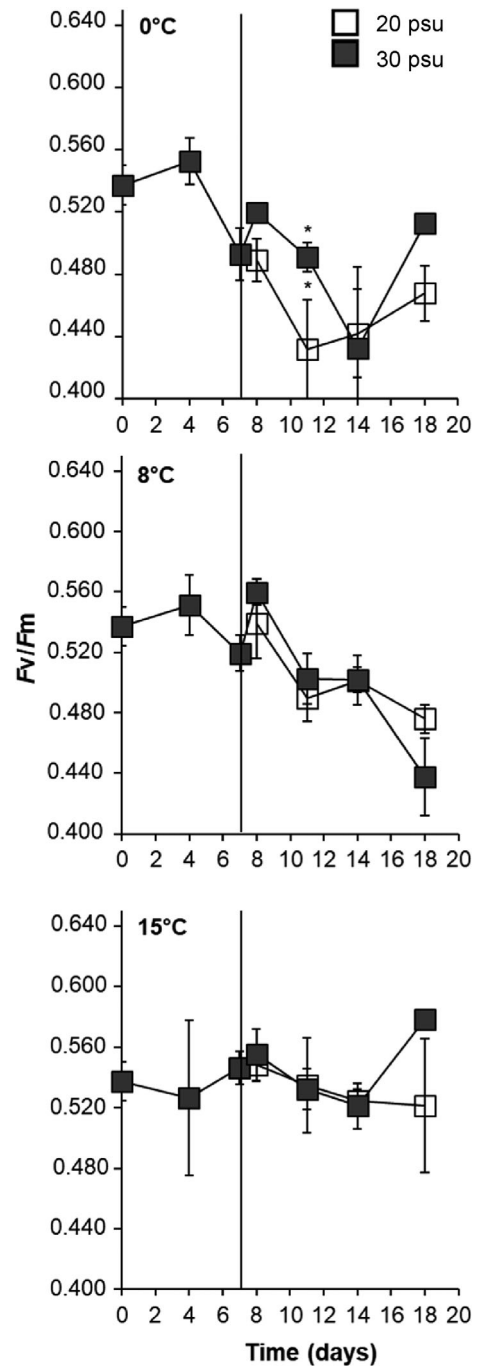


Fig 2. Maximal quantum yield of photosystem II (F_v/F_m) of *Saccharina latissima* from Brittany at day 0, 4, 7, 8, 11, 14 and 18 at 0, 8 and 15°C (mean \pm SD; $n = 5$). Grey boxes mark 30 psu, white boxes 20 psu. On the left, temperature acclimation phase – 7 days at 0, 8, 15°C and control salinity 30 psu; and on the right, hyposalinity exposure phase – sporophytes were exposed to low (20 psu) and control (30 psu) salinity within 0, 8, 15°C for 11 days. Asterisks indicate significant differences between salinities ($P < 0.05$).

At the end of the salinity treatment (day 18) no significant interactions between temperature and salinity were detected in mannitol content but there were significant main effects. Mannitol

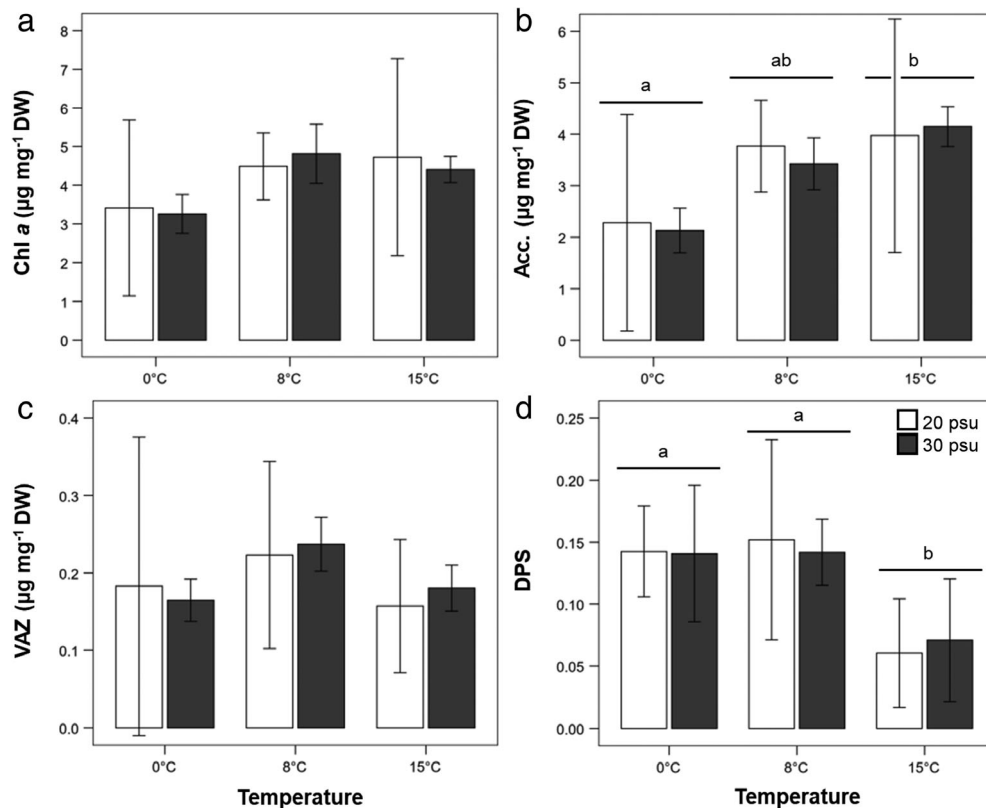


Fig 3. Chlorophyll *a* (Chl *a*) content (a), accessory pigment pool (Acc.) (b), pool of xanthophylls (VAZ) (c) ($\mu\text{g mg}^{-1}$ DW; mean \pm SD; $n = 5$) and de-epoxydation state (DPS) (d) (mean \pm SD; $n = 5$) of *Saccharina latissima* from Brittany after exposure to two salinity conditions (20 psu, 30) at three temperatures (0, 8, 15°C) on day 18. Grey boxes mark 30 psu, white boxes 20 psu. Different lower-case letters indicate significant differences between temperatures ($P < 0.05$). Significant differences were identified for Chl *a* and Acc. after exponential transformation.

content was 550% significantly higher at 0°C than at 8°C and 15°C and significantly lower at low salinity than at the control (203%) (Fig. 4, Appendix S2 – Table 4 in the Supporting Information). Pattern of mannitol variation was similar for days 8 and 11, however, differences between salinity levels were only significant on day 18 (Appendix S4 in the Supporting Information).

On day 18, the interaction between temperature and salinity on C:N ratios could not be tested due to the application of non-parametric test. The C:N ratio was significantly higher at 15°C than at 8°C (11%) and no significant difference to 0°C was observed. There was no significant effect of salinity (Fig. 5, Appendix S2 – Table 4 in the Supporting Information). The variation in the C:N ratio across the experimental period is depicted in the Appendix S5 in the Supporting Information. On days 8 and 11, the C:N ratios were not significantly affected by the interaction of temperature and salinity, however single effects were significant. Overall C:N increased from 0°C to 15°C to 8°C, and overall higher C:N ratio was measured at lower salinities. Total carbon and nitrogen content are available on the Appendixes S6 and S7 in the Supporting Information.

DISCUSSION

The current investigation of *Saccharina latissima* from Brittany revealed that juvenile sporophytes are especially sensitive

to low temperatures of 0°C and to a decreased salinity of 20 psu, both compromising fitness and causing increasing stress while little differences were identified between intermediate (8°C) and high (15°C) temperatures. While all biochemical and physiological parameters significantly responded to temperature, low salinity was also a stress agent but on a lower level and both factors did not interact where testable. Most interesting, and reported here for the first time, is the fivefold increase of mannitol levels in *S. latissima* under low temperature (0°C) which already became activated after a short treatment of 8 days. Comparison with a study with *S. latissima* from an Arctic location (Li *et al.* 2020) which was performed under identical conditions reveals that both populations in general exhibited the same response pattern and cannot thereby be considered as ecotypes in the conditions of this experiment. Nevertheless, differences in initial values, morphology, the time-dependent modulation of some parameters and earlier studies of transcriptomic response, indicate divergence between the two locations, suggesting that ecotypes might be forming and that they might be revealed at the phenotypic level with more extreme stress levels or with different parameters measured. This would be in accordance with the ecotypic differentiation between Arctic and temperate populations (Helgoland, North Sea) already suggested by previous studies (Müller *et al.* 2008; Olischläger *et al.* 2014).

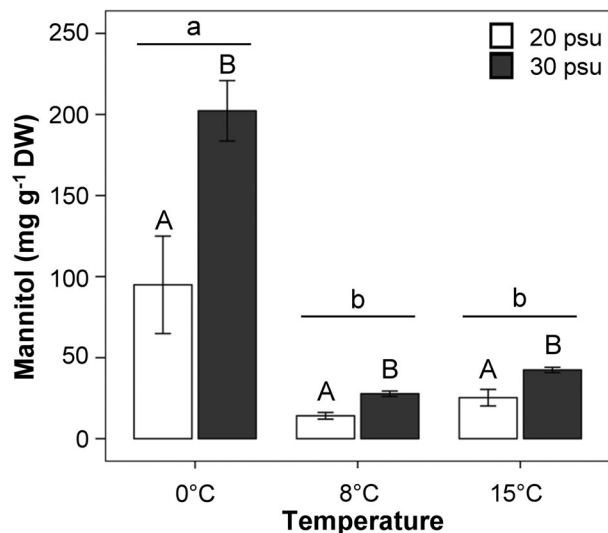


Fig 4. Mannitol content (mg g⁻¹ DW; mean \pm SD; n = 3) of *Saccharina latissima* from Brittany after exposure to two salinity conditions (20 psu, 30) at three temperatures (0, 8, 15°C) on day 18. Grey boxes mark 30 psu, white boxes 20 psu. Different lower-case letters indicate significant differences between temperatures ($P < 0.05$). Different upper-case letters indicate significant differences between salinities ($P < 0.05$). Significant differences were identified after log₁₀ transformation.

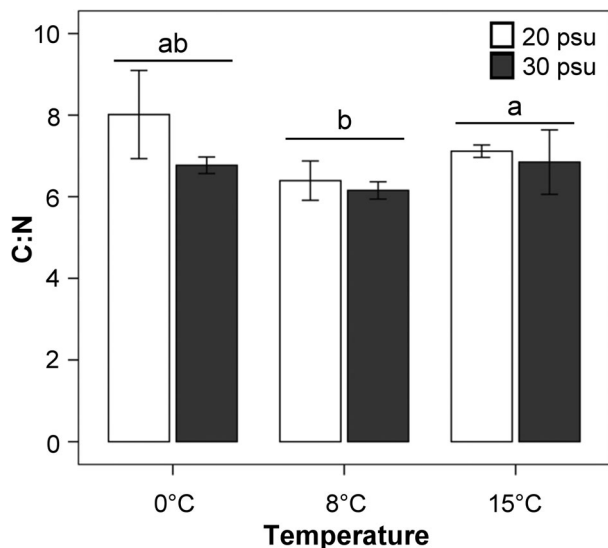


Fig 5. C:N ratio (mean \pm SD; n = 4) of *Saccharina latissima* from Brittany after exposure to two salinity conditions (20 psu, 30) at three temperatures (0, 8, 15°C) on day 18. Grey boxes mark 30 psu, white boxes 20 psu. Different lower-case letters indicate significant differences between temperatures ($P < 0.05$).

Exposure to 0°C indicates compromised photosynthesis at low temperature as effective quantum yield F_v/F_m decreased at several time points and the accessory pigments pool was lowered at day 18; both may explain the overall lower growth of sporophytes at 0°C. The xanthophyll cycle (VAZ and DPS)

is a component of stress response of plants, brown and green seaweeds (Goss & Jakob 2010). The xanthophyll cycle pigment pool (VAZ) was unaffected by temperature in this study. In turn, the Arctic material of *S. latissima* increased its VAZ content at 15°C compared to 0 and 8°C (Li *et al.* 2020). Different modulation of VAZ content between algae originating from different locations (Spitsbergen and Helgoland) has already been reported (Olischläger *et al.* 2017). VAZ was also significantly affected by salinity. Higher VAZ content was already measured at 20 psu after one day of salinity treatment (day 8) but only at 0°C. In contrast, in material from the Arctic this effect was more pronounced as VAZ was higher at 20 psu at all temperatures (Li *et al.* 2020). Furthermore, we observed a decrease of the de-epoxidation state (DPS) at 15°C when compared to 0 and 8°C in algae from Brittany. Similarly, in algae from the Arctic under similar experimental conditions, DPS was significantly higher at 0°C than at 8 and 15°C (Li *et al.* 2020). This is in accordance with Olischläger *et al.* (2017) who observed a decrease in DPS with increasing temperature for sporophytes from the Arctic and Helgoland after 18 days of exposure to a combination of temperature and pCO₂ levels. High DPS values provide protection from photo-oxidative damage by energy dissipation and therefore it is expected to be higher under stressful conditions (Müller *et al.* 2001, Fernández-Marín *et al.* 2011). This was the case at low temperature and at the low salinity treatment applied in this study and thereby supports our hypothesis that a combination of low temperature and low salinities is most stressful for the Brittany population.

Mannitol concentrations were more than 500% higher at 0°C than at 8 and 15°C across the experimental period. This drastic increase at low temperature suggests a cryoprotectant role for mannitol that has not been described in kelps so far. Sugar alcohols, such as mannitol, have been reported as cryoprotectant agents – conferring protection against anti-freezing in several organisms (Elliott *et al.* 2017). In fungi, cold tolerance in polar habitats is positively correlated with sugar alcohol content, among other mechanisms (Robinson 2001). Although the role of mannitol as cryoprotectant has not been described in brown algae so far, a survey of seasonal variation on mannitol concentrations in *Sargassum mangarevense* and *Turbinaria ornata* revealed that the content was higher in winter than summer – 108% in *S. mangarevense* and 164% in *T. ornata* (Zubia *et al.* 2008). Similarly, in a marine ecotype of *Fucus vesiculosus*, mannitol content was higher at 0°C than at 10°C (around 125%), although there were no significant changes in the brackish ecotype (Gylle *et al.* 2009). In the present study, increase in mannitol concentration was considerably higher and it was also a relatively fast response to cold shock, already apparent 8 days after transfer to 0°C. As expected, hypoosmotic conditions led to a decrease in mannitol content compared with control salinity. Mannitol acts as osmolyte and compatible solute in brown macroalgae and thereby maintains the cellular functions under varying salinities (e.g. Kirst 1990; Eggert *et al.* 2007; Diehl *et al.* 2020). Adjustment in osmolyte concentration has been described as the second stage of osmotic acclimation, being a slow and long-term response to changing osmotic conditions, taking up to a few days and being energy demanding (Kirst 1990; Karsten *et al.* 1991b). Mannitol concentration at 20 psu was significantly lower than at 30 psu in all

temperatures after 11 experimental days (day 18), suggesting that adjustment in osmolyte concentrations is a long-term process.

Temperate and tropical field macroalgae feature a mean C:N ratio of 20, with lower values indicating N limitation (Atkinson & Smith 1983). The samples measured in this study had a C:N ratio of approximately 6–10, meaning that the algae did not suffer from N limitation in any experimental condition as expected due to a high NO₃ content supplied by the cultivation medium (Provasoli, Starr & Zeikus 1993). C:N ratios measured in the present work in *S. latissima* were lower than reported before in field Phaeophyceae (Peters *et al.* 2005; Scheschonk *et al.* 2019). Several studies report increasing C:N ratios at sub-optimal temperatures on brown macroalgae (e.g. Gordillo *et al.* 2006; Graiff *et al.* 2015). Temperature variation induces changes in the enzymatic reaction, affecting for instance nutrient uptake and assimilation (Hurd *et al.* 2014). We could not detect distinct impacts of temperature or salinity on the C:N ratio on day 18. Though the variation is marginal, significant differences in C:N ratio were detected between 8 and 15°C, possibly since our sporophytes were grown at 8°C.

Genomic basis of stress response to salinity and temperature variation

Transcriptomic analyses of material from the same experiment as reported here, revealed that after 24 h of exposure to low salinity (day 8) extensive metabolic reprogramming took place in Arctic and Brittany *S. latissima* material (Monteiro *et al.* 2019). Metabolic pathways involved included photosynthesis, pigment synthesis, transport, signaling, cell wall synthesis and reorganization and stress related enzymes. Interestingly, the overall extensive repression of photosynthesis related DEGs after 1 day of salinity treatment was not mirrored in the pigment contents and quantum yield of the sporophytes (this study; Monteiro *et al.* 2019; Li *et al.* 2020). Although no data on gene expression is available for day 18, we assume that effects at the physiological level are revealed later than at the transcriptomic level as has been reported earlier (Heinrich *et al.* 2015; Iñiguez *et al.* 2017). An alternative explanation is that algae were able to acclimate to short-term hyposalinity (one day) by metabolic reorganization that stabilized physiological responses. While this may be valid for explaining short-term responses, long-term regulation possibly is different as the algae were no longer able to withstand the effects of low salinity after 11 days of exposure and growth was compromised. An ameliorating effect of high temperature was observed at the transcriptomic level for *S. latissima* sporophytes from both Brittany and the Arctic (Monteiro *et al.* 2019). At 15°C, low salinity drove less transcriptomic changes than at the lower temperatures of 0 and 8°C. In contrast, interactive effects at the photo-physiological level (*Fv/Fm* and pigment content) were less prominent within material of both populations (this study and Li *et al.* 2020).

Differences driven by geographical variation

The existence of ecotypes has been described for *S. latissima* across its latitudinal range (e.g. Gerard & Du Bois 1988;

Müller *et al.* 2008). An ecotype is defined to perform better at the local conditions than another population from a distant location with other local environmental factors (Kawecki & Ebert 2004). Therefore, we compared data gathered here for material from Roscoff, Brittany to Arctic material from Spitsbergen presented in Li *et al.* (2020). Algae from both locations were cultivated at similar conditions from the gametophyte stage and were exposed to an identical experimental design. Samples from the Arctic grew faster than algae from Brittany in all conditions. For both locations, growth, pigment content, *Fv/Fm* was generally higher at higher temperatures than at 0°C. Overall, low salinity led to a decrease in growth and *Fv/Fm* and an increase in xanthophyll cycle pigments VAZ in algae from both locations significantly at certain time points. Therefore, different to our hypothesis, we do not observe a higher growth rate and physiological performance of *S. latissima* from the Arctic at the local conditions (low salinity and low temperature) compared to material from Brittany. Hence, ecotypic differentiation cannot be implied from the results of biochemical and physiological parameters measured during this experiment. Nonetheless, we observed differences between sporophytes from both locations in several parameters, at the end of the cultivation phase on day 0 - namely fresh weight, pigment content, and *Fv/Fm* (data not shown). Similarly, differences in biochemical composition and fresh weight in response to the same level of temperature (10°C) have been previously reported between a temperate and Arctic population (Olischläger *et al.* 2014). In addition, morphology differed considerably between algae from Brittany and the Arctic indicating phenotypes. Sporophytes from the Arctic were narrower and longer while algae from Brittany were wider and shorter. This diverging morphology was also described between sporophytes of *S. latissima* from a glacially-influenced site and an oceanic site in Alaska, USA (Spurkland & Iken 2012). Moreover, even though similar trends in pigment content variation were observed for both locations, there were differences across exposure time. A stronger short-term response was observed in sporophytes from Brittany than from Spitsbergen. On day 18, only DPS and Acc. were significantly modulated in algae from both locations; however, DPS was significantly higher at low salinity only in sporophytes from the Arctic. Moreover, a significant decrease in growth due to low salinity was evident already on day 11 in algae from Brittany but only on day 18 for algae from the Arctic. Furthermore, at the transcriptomic level, short-term transcriptomic responses between sporophytes of both locations diverged both in magnitude and metabolic pathways involved that correlate to a certain extent with local conditions (Monteiro *et al.* 2019). Therefore, we suggest that ecotypic differentiation is already taking place and might in future be revealed at the physiological and biochemical level by studies targeting extreme abiotic factor levels and/or longer exposure times.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Experimental design and parameters measured during the temperature and salinity experiment in sporophytes of *Saccharina latissima*. In laboratory, sporophytes were cultivated at 8°C and 30 psu. At the start of the experiment, they were acclimated for seven days at 0, 8 and 15°C. After seven days, they were exposed to a low salinity of 20 psu for eleven days, resulting in a total of 18 experimental days. Sampling days are indicated for pigment content, growth, maximal

quantum yield of photosystem II (F_v/F_m), mannitol content; carbon (C) and nitrogen (N) contents and carbon to nitrogen ratio (C:N).

Appendix S2. Results of the statistical tests applied to the parameters F_v/F_m , fresh weight, pigments, mannitol and C:N.

Appendix S3. Chlorophyll *a* (Chl *a*) content (A), accessory pigment pool (Acc.) (B), pool of xanthophylls (VAZ) (C) ($\mu\text{g mg}^{-1}$ DW; mean \pm SD; $n = 5$) and de-epoxydation state (DPS) (D) (mean \pm SD; $n = 5$) of *Saccharina latissima* from Brittany after exposure to two salinity conditions (20 psu, 30) and three temperatures (0, 8, 15°C) on day 8. Grey boxes mark 30 psu, white boxes 20 psu. Different lower-case letters indicate significant differences between temperatures ($P < 0.05$). Significant differences were identified for Chl *a* and Acc. after exponential transformation.

Appendix S4. Mannitol content (mg g^{-1} DW; mean \pm SD; $n = 3$) of *Saccharina latissima* from Brittany after exposure to two salinity conditions (20 psu, 30) and three temperatures (0, 8, 15°C) across experimental days 8, 11 and 18. Different lower-case letters indicate significant differences between

temperatures ($P < 0.05$). Different upper-case letters indicate significant differences between salinities ($P < 0.05$). For day 18, significant differences were identified on day 18 after log₁₀ transformation.

Appendix S5. C:N ratio (mean \pm SD; $n = 4$) of *Saccharina latissima* from Brittany after exposure to two salinity conditions (20 psu, 30) and three temperatures (0, 8, 15°C) across experimental days 8, 11 and 18. Different lower-case letters indicate significant differences between temperatures ($P < 0.05$). Different upper-case letters indicate significant differences between salinities ($P < 0.05$). For days 8 and 11, significant differences were identified at day 8 and 11 after log₁₀ transformation.

Appendix S6. Carbon content (mg g^{-1} DW; mean \pm SD; $n = 4$) of *Saccharina latissima* from Brittany after exposure to two salinity conditions (20 psu, 30) and three temperatures (0, 8, 15°C) across experimental days 8, 11 and 18.

Appendix S7. Nitrogen content (mg g^{-1} DW; mean \pm SD; $n = 4$) of *Saccharina latissima* from Brittany after exposure to two salinity conditions (20 psu, 30) and three temperatures (0, 8, 15°C) across experimental days 8, 11 and 18.