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Transcriptional bases of acclimation in the kelp, *Saccharina latissima*, from gametophytes to sporophytes

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Photo by Arvid Åsen

"I can only compare these great aquatic forests... with the terrestrial ones in the intertropical forests. Yet if in any country, a forest was destroyed, I do not believe nearly so many species of animals would perish as would here, from the destruction of the kelp. Amidst the leaves of this plant, numerous species of fish live, which nowhere else could find food or shelter; with their destruction the numerous cormorants and fishing birds, the otters, seals, and porpoise, would soon perish."

Charles Darwin
1 June 1834, Tierra del Fuego, Chile

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Summary

Kelps, algae of the order Laminariales, are important ecosystem engineers in temperate to Arctic coastal waters. They form marine forests that support complex communities by providing habitat for several taxa, they provide coastal defense by protecting against erosion and contribute significantly to climate change mitigation and adaptation by acting as carbon sink. However, these marine forests are threatened by impacts of anthropogenic-driven climate change.

The sugar kelp *Saccharina latissima* is widely distributed in the North Atlantic. Apart from its significant ecological value, *S. latissima* is one of the key species targeted in seaweed cultivation in the North Atlantic, with several industrial applications. Along its distributional range it experiences high variability of abiotic factors that are expected to modulate fitness and survival. To what extent *S. latissima* copes with this environmental variation either by phenotypic plasticity or local adaptation has not yet been resolved. In this thesis the effect of the abiotic drivers temperature and salinity on the performance of sporophytes of *S. latissima* is explored. These two factors substantially influence *S. latissima* growth and survival and are both expected to change under future climate change scenarios. However, impacts of environmental variation often differ between macroscopic sporophytes and microscopic early life history stages. The resilience of kelp gametophytes to environmental stress has been poorly explored so far, although they are expected to significantly determine recruitment and adult population dynamics. Hence, a better understanding of the acclimation mechanisms taking place in *S. latissima* across its life history stages is necessary to predict emerging environmental pressures, potential range shifts and, thus, future distributions.

The combined effects of temperature and salinity on physiology, photosynthetic performance and gene expression on young sporophytes of *S. latissima* from Spitsbergen (Arctic) were investigated (Chapter 3). Young sporophytes were acclimated to three temperatures (0 °C, 8 °C and 15 °C) for one week. Then, algae were exposed to low salinity (S_A 20) and control salinity (S_A 30) at each temperature. High temperature (15 °C) promoted growth and pigment composition. Low salinity significantly impacted growth at the end of the 11 days salinity exposure. In parallel, low salinity drove considerable reprogramming at the gene expression level. Several pathways were involved in the acclimation to salinity, including photosynthesis, pigment synthesis, carbon metabolism, stress related pathways and cell wall adjustment. Results suggest that *S. latissima* currently experiences sub-optimal temperatures in the Arctic and therefore will benefit from increasing temperatures due to climate change. However, low salinities due to increased freshwater input might compromise performance, as a likely scenario for Arctic fjord ecosystems.

A genome-wide transcriptomic analysis of geographical variation influence on the response to multiple stressors was conducted, for the first time in brown algae. Sporophytes of *S. latissima* from Roscoff, Brittany, temperate Atlantic and Spitsbergen, Norway, Arctic were raised under identical controlled conditions and subjected to the same temperature and salinity levels (Chapter 4). Both isolates revealed distinct transcriptomic profiles between the two locations. The expression of several genes related to cell wall and cytoskeleton organization differed between sporophytes from Roscoff and Spitsbergen

which could be associated with the distinct morphologies of the two isolates. The combination of low salinity and low temperature provoked a stronger response in temperate than in Arctic sporophytes. This response comprised a strong down-regulation of photosynthesis and pigment synthesis related genes. Taken together, our results suggest that a higher investment in transcriptomic reprogramming is required in sporophytes from Roscoff compared to Spitsbergen to acclimate to low temperature and low salinity. This can probably be explained by the fact that sporophytes from the Arctic often experience low temperature and hyposalinity and that in turn these are uncommon stressors to sporophytes from Roscoff.

The effects of temperature on transcriptomic profiles of vegetative gametophytes of *S. latissima* were investigated, for the first time, by applying three temperature levels (4 °C, 12 °C and 20 °C) to both males and females for two weeks. The experiment revealed a female-biased gene expression that was increased with rising temperature. Induction of carbohydrate, lipid and nucleic acid metabolism together with energy production in female gametophytes was consistent with an investment in cell growth. Concomitantly, up-regulation of signaling, cytoskeleton and organelle organization related genes in male gametophytes was reflected by high cell division activity. Gene expression profiles suggest that while vegetative, gametophyte cells are still metabolically active and they prepare for maturation in order to synchronize gamete release and ensure fertilization. In addition, the increase in stress related pathways in female gametophytes compared to males at 20 °C indicate that females are more sensitive to heat, which might have repercussions for reproductive success and compromise persistence of *S. latissima* under increasing temperatures.

In conclusion, the study revealed novel insights into the molecular mechanisms of acclimation and adaptive responses of a keystone, but non-model species, for which only limited genomic information has been available previously. It also became evident that transcriptomic data do not necessarily match observations made on the physiological level, but are likely to reflect emerging constraints to cellular function and overall individual performance at an early stage. Furthermore, the differences in response towards environmental drivers across different latitudes, life history stages and sexes emphasize the need for integrative approaches in order to facilitate predictions on species performance in natural as well as aquaculture systems in the face of large-scale environmental change.

Zusammenfassung

Braunalgen aus der Ordnung Laminariales sind wichtige Ökosystem-Ingenieure in gemäßigten bis arktischen Küstengewässern. Sie bilden Unterwasser-Wälder, welche komplexe Lebens-Gemeinschaften ausbilden, indem sie Lebensraum für mehrere Taxa bieten, sie dienen dem Küstenschutz und tragen durch ihre Funktion als Kohlenstoffsenke wesentlich zur Minderung und Anpassung an den Klimawandel bei. Diese Ökosysteme sind jedoch zunehmend durch die Auswirkungen des anthropogen bedingten Klimawandels bedroht.

Der Zuckertang *Saccharina latissima* ist im Nordatlantik weit verbreitet. Entlang ihres Verteilungsgebietes ist die Alge einer großen Variabilität abiotischer Faktoren ausgesetzt, welche die individuelle Fitness und das Überleben der Art bestimmen. Inwieweit phänotypische Plastizität oder lokale Adaptation die Grundlage für den großen Toleranzbereich von *S. latissima* bildet, ist noch nicht geklärt. In dieser Arbeit werden die Auswirkungen der abiotischen Faktoren Temperatur und Salzgehalt auf die Leistung der Sporophyten von *S. latissima* untersucht. Diese beiden Faktoren beeinflussen das Wachstum und das Überleben von *S. latissima* erheblich und werden sich laut der Szenarien des Klimawandels signifikant verändern. Allerdings unterscheiden sich die Auswirkungen von Umweltvariationen auf Sporophyten einer Algenart oft von den Reaktionen der frühen Entwicklungsstadien. So wurde die Toleranz der Gametophyten gegen Umweltstress bislang nur unzureichend untersucht, obwohl erwartet wird, dass diese die Populationsdynamik erheblich mitbestimmen. Daher ist ein besseres Verständnis der Anpassungsmechanismen, die in den verschiedenen Entwicklungsphasen von *S. latissima* stattfinden, notwendig, um auftretende Umweltbelastungen und zukünftige Verbreitungsgebiete vorherzusagen.

Die kombinierten Auswirkungen von Temperatur und Salzgehalt auf Physiologie, Photosynthese und Genexpression auf junge Sporophyten von *S. latissima* aus Spitzbergen (Arktis) wurden untersucht (Kapitel 3). Junge Sporophyten wurden eine Woche lang an drei Temperaturen (0° C, 8° C und 15° C) akklimatisiert. Anschließend wurden die Algen bei jeder Temperatur einem niedrigen Salzgehalt (S_A 20) und einer Kontroll-Salinität (S_A 30) ausgesetzt. Hohe Temperaturen (15° C) förderten das Wachstum und die Pigmentzusammensetzung. Ein niedriger Salzgehalt beeinträchtigte das Wachstum am Ende der 11-tägigen Exposition signifikant. Parallel dazu führte der niedrige Salzgehalt zu einer erheblichen Umprogrammierung auf der Ebene der Genexpression. Mehrere Stoffwechselwege waren an der Akklimatisierung an den Salzgehalt beteiligt, darunter Photosynthese, Pigmentsynthese, Kohlenstoffstoffwechsel, Stressabwehr und Zellwandsynthese. Die Ergebnisse deuten darauf hin, dass *S. latissima* derzeit unter suboptimalen Temperaturen in der Arktis vorkommt und daher dort von steigenden Temperaturen aufgrund des Klimawandels profitieren wird. Niedrige Salinitäten aufgrund des erhöhten Süßwassereintrags, welcher für die Ökosysteme der arktischen Fjorde wahrscheinlich ist, könnten jedoch *S. latissima* beeinträchtigen.

Um den Einfluss der geografischen Verbreitung auf die Stresstoleranz von *S. latissima* zu bewerten, wurden Sporophyten aus Roscoff (Bretagne, gemäßigter Atlantik) und Spitzbergen (Norwegen, Arktis) unter den gleichen kontrollierten Bedingungen angezogen und den gleichen Temperatur- und Salinitätsbedingungen ausgesetzt (Kapitel 4). Beide Isolate zeigte unterschiedliche Transkriptionsprofile

zwischen den beiden Standorten. Die Expression mehrerer Gene, die die Funktionalität der Zellwand und des Zytoskeletts steuern, unterschied sich zwischen Sporophyten von Roscoff und Spitzbergen. Diese Unterschiede scheinen mit den unterschiedlichen Morphologien der beiden Isolate assoziiert zu sein. Die Kombination aus niedrigem Salzgehalt und niedriger Temperatur rief eine stärkere Reaktion in den Sporophyten aus gemäßigten Regionen hervor, als bei arktischen Sporophyten. Diese Reizantwort umfasste eine starke Herunterregulation der Gene mit Funktion in der Photosynthese und der Pigmentsynthese. Zusammenfassend deuten die Ergebnisse darauf hin, dass bei Sporophyten aus Roscoff im Vergleich zu Spitzbergen eine höhere Investition in transkriptomische Umprogrammierung erforderlich ist, um sich an niedrige Temperaturen und niedrige Salzgehalte anzupassen. Dies lässt sich wahrscheinlich dadurch erklären, dass Sporophyten aus der Arktis oft niedrigen Temperaturen und Hyposalinität ausgesetzt sind und dass es sich andererseits dabei um Stressoren handelt, die für Sporophyten aus Roscoff ungewöhnlich sind.

Der Einfluss der Temperatur auf die vegetativen Gametophyten von *S. latissima* wurde untersucht, indem männliche und weibliche Gametophyten für zwei Wochen drei Temperaturstufen (4° C, 12° C und 20° C) exponiert wurden. Im Laufe des Experiments konnte eine Expression spezifisch weiblicher Gene beobachtet werden, die mit steigender Temperatur erhöht wurde. Die Induktion des Energie-, Kohlenhydrat-, Lipid- und Nukleinsäurestoffwechsels in weiblichen Gametophyten spiegelte sich in der Investition in das Zellwachstum wider. Gleichzeitig wurde die Heraufregulation von Genen mit Funktion in der Signal-, Zytoskelett- und Organellenorganisation in männlichen Gametophyten durch eine hohe Zellteilungsaktivität reflektiert. Die beobachteten Genexpressionsprofile deuten darauf hin, dass vegetative Gametophytenzellen metabolisch aktiv sind, um sich auf die Reifung vorzubereiten und die Freisetzung von Gameten zu synchronisieren, um so die Befruchtung sicherzustellen.

Folglich lieferte diese Studie neuartige Erkenntnisse über die molekularen Mechanismen der Akklimatisierung und die adaptiven Reaktionen einer ökologischen Schlüsselart, für die bislang allerdings nur begrenzte genomische Informationen verfügbar waren. Die Studie zeigt auch, dass transkriptomische Daten nicht unbedingt mit Beobachtungen auf physiologischer Ebene übereinstimmen, aber aufkommende Einschränkungen der Zellfunktion und der individuellen Leistung eines Organismus schon in einem frühen Stadium der Reizeinwirkung widerspiegeln können. Darüber hinaus unterstreichen die Unterschiede in der Reaktion von Individuen von verschiedener geographischer Herkunft, Entwicklungsstadium und Geschlecht die Notwendigkeit integrativer Ansätze, um Vorhersagen über die Leistung von Arten in natürlichen Ökosystemen und in der Aquakultur angesichts großer Umweltveränderungen zu erleichtern.

Résumé

Les algues brunes, membres de l'ordre des Laminariales, sont des espèces-ingénieures dans la zone côtière des océans tempérés jusqu'à l'Arctique. Elles forment des forêts sous-marines qui supportent des communautés complexes en fournissant des habitats à plusieurs espèces, elles assurent une protection des côtes contre l'érosion et contribuent de manière significative à l'atténuation du changement climatique en agissant comme des puits de carbone. Cependant, ces forêts marines sont menacées par les impacts du changement climatique d'origine anthropique.

Saccharina latissima est largement distribué dans l'Atlantique Nord. Tout au long de son aire de distribution, elle est confrontée à une grande variabilité des facteurs abiotiques qui devraient moduler sa valeur sélective et sa survie. Comment *S. latissima* s'acclimate par des phénomènes de plasticité phénotypiques ou est adaptée localement à ces facteurs environnementaux est une question encore non résolue. Dans cette thèse, j'explore les effets des facteurs abiotiques, température et salinité, sur la performance des sporophytes de *S. latissima*. Ces facteurs influencent de manière substantielle la croissance et la survie de *S. latissima* et sont tous deux impactés dans les scénarios de changements climatiques futurs. Leurs impacts sur les sporophytes diffèrent cependant souvent des effets sur les premiers stades du cycle de vie. La résilience des gamétophytes de Laminariales aux stress environnementaux a été peu explorée jusqu'à présent, bien qu'elle doive avoir un impact significatif sur la dynamique du recrutement sporophytique et des populations adultes. Par conséquent, une meilleure compréhension des mécanismes d'acclimatation chez *S. latissima* est nécessaire pour prévoir sa distribution future.

Les effets combinés de la température et de la salinité sur la physiologie, la photosynthèse et l'expression des gènes chez les jeunes sporophytes de *S. latissima* issus de Spitsberg (Arctique) ont été étudiés en laboratoire et les résultats sont présentés dans le chapitre 3. Les jeunes sporophytes ont été acclimatés à trois températures (0 °C, 8 °C et 15 °C) pendant une semaine. Ensuite, les algues ont été exposées à une salinité réduite (S_A 20) et à une salinité témoin (S_A 30), pour chaque température de culture. La température élevée (15 °C) stimule la croissance et la composition des pigments. La salinité réduite a un impact significatif sur la croissance à la fin de l'exposition de 11 jours. En parallèle, une salinité réduite a entraîné une reprogrammation considérable au niveau de l'expression des gènes. Ces changements concernent plusieurs voies métaboliques vraisemblablement impliquées dans l'acclimatation à la salinité, comme la photosynthèse, la synthèse de pigments, le métabolisme du carbone, les voies liées au stress et à l'ajustement de la paroi cellulaire. Nos résultats suggèrent que *S. latissima* est actuellement exposée à des températures sous-optimales dans l'Arctique et bénéficiera donc de la hausse des températures. Cependant, les faibles salinités dues à un apport augmenté d'eau douce pourraient compromettre ses performances.

Pour évaluer l'effet de la localisation géographique sur la réponse aux multiples agents stressants chez *S. latissima*, les sporophytes originaires de Roscoff (Bretagne), de la région tempérée de l'Atlantique, et de Spitzberg (Norvège, Arctique) ont été élevés dans les mêmes conditions et soumis aux mêmes niveaux de température et de salinité (Chapitre 4). Nous avons trouvé des profils transcriptomiques distincts entre les deux sites. Plusieurs gènes importants pour la paroi cellulaire et dans l'organisation du cytosquelette montrent des expressions différentes entre les sporophytes de Roscoff et du Spitzbergen,

qui sont potentiellement associés à des morphologies distinctes. La combinaison d'une faible salinité et d'une basse température a provoqué une régulation transcriptomique plus forte chez les sporophytes des régions tempérées que chez les sporophytes arctiques. Cette réponse entraîne une forte répression des gènes liés à la photosynthèse et à la synthèse de pigments. Dans l'ensemble, nos résultats suggèrent qu'un investissement plus important dans la reprogrammation transcriptomique est requis chez les sporophytes de Roscoff par rapport à ceux ici de Spitzberg afin de s'acclimater à une température basse et à une salinité faible. Cela peut probablement s'expliquer par le fait que les sporophytes en Arctique sont soumis fréquemment à des basses températures et à une hyposalinité alors que ces agents stressants sont rares pour les sporophytes de Roscoff.

Les effets de la température sur les profils transcriptomiques des gamétophytes végétatifs de *S. latissima* ont été étudiés, pour la première fois, à trois températures (4 ° C, 12 ° C et 20 ° C), à la fois sur les mâles et les femelles, pendant deux semaines. Les résultats de cette expérience ont montré que l'expression des gènes chez les femelles s'accroit avec l'augmentation de la température. L'induction du métabolisme des glucides, des lipides, des acides nucléiques et la production d'énergie chez les gamétophytes femelles correspondaient à un investissement dans la croissance cellulaire. De façon concomitante, une hausse de l'expression des gènes de gamétophytes males liés à la signalisation, au cytosquelette et aux organites suggère une activité élevée de division cellulaire. Les profils transcriptomiques suggèrent que bien que végétatives, les cellules de gamétophytes sont encore actives au niveau métabolique et se préparent pour la maturation, afin de synchroniser la libération des gamètes et d'assurer la fécondation. De plus, l'induction des voies métaboliques liées au stress chez les gamétophytes femelles par rapport aux mâles, à 20 ° C, indique que les femelles sont plus sensibles à l'augmentation de température, avec des répercussions possibles sur le succès reproducteur et la persistance de *S. latissima* en réponse au réchauffement climatique.

En conclusion, cette étude est à l'origine de nouvelles connaissances sur les mécanismes moléculaires d'acclimatation et les réponses adaptatives d'une espèce clé, mais non modèle, pour laquelle les informations génomiques disponibles étaient encore limitées auparavant. Il est également devenu évident que les données transcriptomiques ne correspondent pas nécessairement aux observations au niveau physiologique, mais sont susceptibles de refléter, à un stade précoce, les contraintes émergentes en lien avec les fonctions cellulaires et la performance globale de l'individu. De plus, les différences de réponse aux facteurs environnementaux à travers différentes latitudes, étapes du cycle de vie et sexes soulignent la nécessité d'approches intégratives afin de faciliter les prédictions sur la performance et la résilience des espèces dans les systèmes naturels et aquacoles face aux changements environnementaux importantes.

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1   General introduction

1.1   Ecological relevance of kelps

Seaweeds (or marine macroalgae) are photosynthetic organisms that form extensive habitats along rocky coastlines and provide a number of vital ecosystem functions (Dijkstra et al., 2012). Brown seaweeds belong to the phylum Ochrophyta and are distributed worldwide. Kelps are the representatives of the order Laminariales in the strict taxonomical sense, however, the term often includes genera from the order of Fucales with similar ecological functions as Laminariales, such as *Durvillaea* and *Himanthothallus* (Dayton, 1985). Kelps are among the largest seaweed and are important components of benthic temperate to Arctic ecosystems (Teagle et al., 2017). They contribute greatly to the near-shore primary production and support food-webs through direct consumption by herbivores, but mostly through detritus input (Bartsch et al., 2008; Wiencke and Bischof, 2012). Moreover, they contribute to coastal defense by wave damping and attenuation, an ecosystem service that will be highly required in the future due to climate-change induced sea level rise and higher storm frequency (Løvås and Tørum, 2001; Smale et al., 2013). Some species are considered ecosystem engineers as they provide a complex habitat for several other organisms such as fish and crustaceans (Figure 1, Christie et al., 2009). These habitats might function as nurseries (Holbrook et al., 1990) and even as shelter for large charismatic species as illustrated by the case of the giant kelp *Macrocystis pyrifera* off California and the associated sea otters (Foster and Schiel, 1988). Therefore kelps have a disproportionate value to the ecosystem and changes in their distribution and abundance might have cascading effects throughout the food web (Graham, 2004; Koenigs et al., 2015; Paar et al., 2019). Also, as photosynthetic organisms, they may contribute to climate change mitigation and adaptation (Duarte et al., 2017) when acting as carbon sink (Krause-Jensen and Duarte, 2016). The potential for carbon sequestration of seaweeds surpasses that of other marine macrophytes, such as seagrasses and mangroves (Krause-Jensen and Duarte, 2016). Evidence for widespread macroalgal export to the deep-sea has recently been revealed, with the contribution of Laminariales increasing at depths 3,000 to 4,000 m (Ortega et al., 2019).
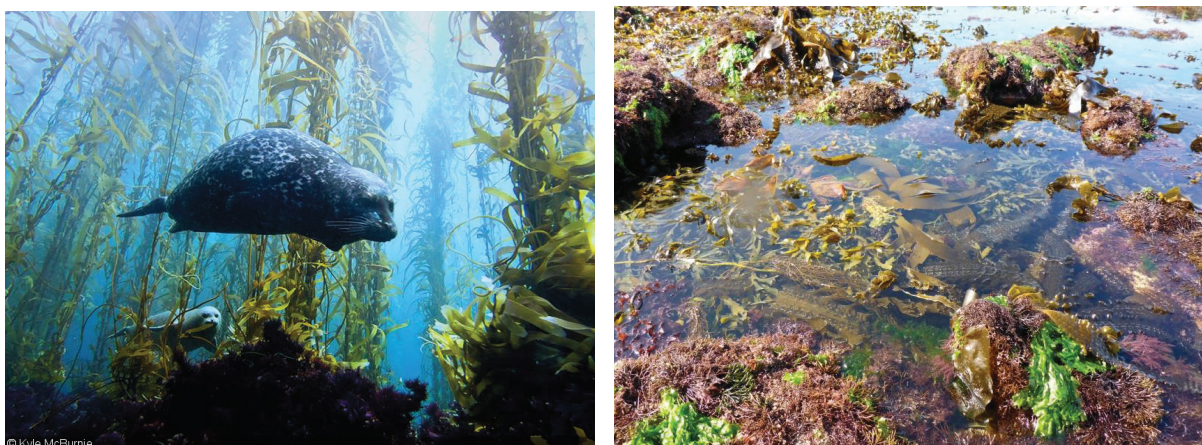
Figure 1 On the left, a giant kelp forest in San Diego, California with a visiting harbor seal. Photo by Kyle Mcburnie. On the right: macroalgal forest at low tide in Roscoff, Brittany, France. Several species can be seen in this assemblage, including *Saccharina latissima* at the right bottom. Photo by Nora Diehl

1.2   Economic value of kelps

Kelps hold increasing economic value as they can be exploited by several industries. Kelp compounds such as alginate, mannitol, laminarin, iodine, pigments, phenolics and lipids have diverse industrial applications (Stengel et al., 2011). Namely, alginate is used in food processing and textile industries and has been commercially extracted from seaweeds for decades (Tseng, 2001; Bixler and Porse, 2011). Recently, research efforts have focused on uncovering bioactive compounds that can improve human health. Antioxidant, antiviral, anticancer and anticoagulant effects in *in vitro* and animal trials have been reported (Hafting et al., 2015; Sanjeewa et al., 2017). Moreover, seaweed compounds can be used as raw material for cosmeceutical, functional food, nutraceutical, and pharmaceutical production (Jiménez-Escrig et al., 2012; Wells et al., 2017; Afonso et al., 2019).

Seaweeds have been traditionally harvested in several coastal countries (Frangoudes, 2011) but currently they are mostly produced by aquaculture (96.5%) in a total of 31 million tons of seaweeds per year (FAO, 2018) a large part of this being *Saccharina japonica*. Particularly, cultivation of the sugar kelp *Saccharina latissima* (formerly *Laminaria saccharin*a L. (Lamour)) (Lane et al., 2006) has been growing in European waters (Buck and Buchholz, 2004; Sanderson et al., 2012; Azevedo et al., 2016; Freitas et al., 2016; Stévant et al., 2017) as its fast growth and high content of carbohydrates make it a suitable choice for cultivation with applications in food, feed and biofuels industries (Kraan, 2013; Manns et al., 2017; Zhang and Thomsen, 2019). Namely, the application of sulfated polysaccharides of *S. latissima* in pharmaceutical industry is promising (Ehrig and Alban, 2015). Furthermore, the sugar alcohol mannitol has several uses in pharmaceutical, paint, leather, paper and plastics industries (Holdt and Kraan, 2011; Scullin et al., 2015). Fermentable sugars of *S. latissima* (mannitol, laminarin, and alginate) have been investigated for the production of bioethanol (Adams et al., 2008; Lopez-Contreras et al., 2016). Successful production of biofuels would offer a sustainable alternative to fossil fuels and contribute to climate change mitigation. The consumption of seaweeds in Europe has also been rising as a result of popularity of seaweed-based Asian dishes but also on an interest in healthy, sustainable food products. Therefore, efforts have been made to improve nutritional value and quality of *S. latissima* to increase its appeal to the food industry (Chapman et al., 2015; Stévant, 2019). Moreover, cultivation of kelps such as *S. latissima* may serve as a bio-mitigation measure, extracting nutrients from coastal systems. They might remove excessive nutrients from eutrophic waters and/or reduce nutrient input into the ecosystem by fish farming in an Integrated Multi-Trophic Aquaculture (IMTA) system (Kim et al., 2015; Marinho et al., 2015; Bruhn et al., 2016).

Several studies have revealed that seasons, geographical variation and site-specific variation of abiotic factors significantly affect chemical composition, hence impacting the content of highly valuable compounds (e.g. Schiener et al., 2015; Fernandes et al., 2016; Bruhn et al., 2017). Therefore future research should target the range of environmental conditions (e.g. temperature, salinity, exposure) that allow optimization of *S. latissima* production to the requirements of the industries mentioned above.

1.3   Life cycle of kelps

Kelps (order Laminariales) display a haplodiplontic (also termed haploid-diploid) heteromorphic life cycle (Figure 2, Hurd et al., 2014a). Moreover, they are dioecious: male and female individuals only occur during the haploid phase (spores, gametophytes and gametes). Mature macroscopic sporophytes (2n) produce spores (=zoospores, = meiospores) (n) by meiosis that in *Saccharina latissima* accumulate at the surface of the thallus producing a sorus (Lüning, 1988). The spores are released into the water and quickly attach to an available substrate. Once they germinate they develop into female and male gametophytes that display sexually dimorphic traits. Female gametophyte cells are larger and rounder while male gametophytes cells are smaller and tend to form filaments with more cells (Lüning and Neushul, 1978; Destombe and Oppliger, 2011). Once these gametophytes mature, male reproductive structures called antheridia produce male gametes (sperm), and respectively female reproductive structures (oogonia) produce eggs. Upon encounter, egg and sperm will fertilize and produce a zygote that will grow into a young sporophyte. Kelp gametes are oogamous, meaning egg cells are significantly larger than male gametes, and are non-motile as opposed to male gametes (Luthringer et al., 2014). While sporophytes are macroscopic and divided into morphological structures such as holdfast, stipe, lamina and blade (also termed rhizoid, cauloid and phylloid, Staufenberger et al., 2008); spores, gametophytes and gametes are microscopic structures often referred to as early life history stages (Charrier et al., 2012; Hurd et al., 2014c). As free-living stages, spores and gametes are the phases that allow for dispersal, even though dispersal is limited in kelps and therefore spores tend to settle within close proximity to parent sporophytes (Schiel and Foster, 2006).
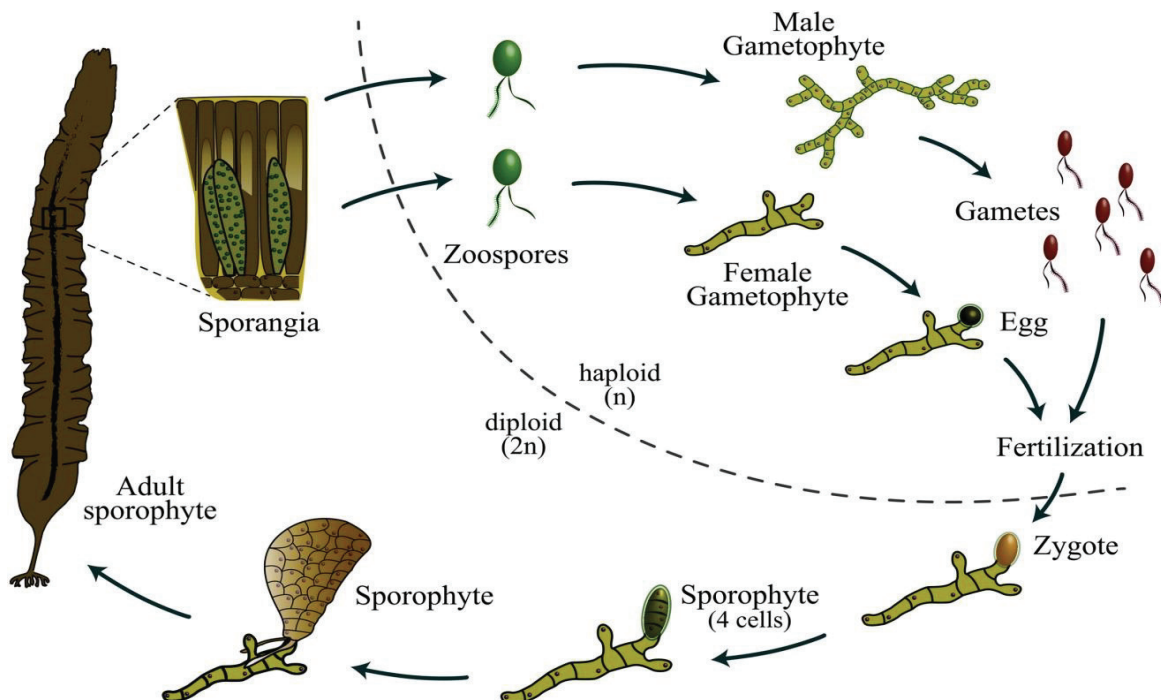
Figure 2 Schematic representation of the life cycle of kelps. The adult sporophyte is a representation of *Saccharina latissima.* From Visch et al. (2019)

Due to technical and sampling constraints microscopic stages are often overlooked in research, which results in most studies failing to include the whole life cycle and as so conclusions on the species survival and performance may be misleading. Moreover, the early developmental stages have been identified as the most vulnerable to a variety of environmental perturbations (Dring et al., 1996; Coelho et al., 2000; Roleda et al., 2007). On the contrary, other studies indicate higher sensitivity of sporophytes to temperature changes. Gametophytes of several kelp species exhibit higher upper thermal tolerance of about 3-4 °C than sporophytes (tom Dieck, 1993) and photosynthetic efficiency of gametophytes of the kelp *Alaria crassifolia* was less affected by low temperature than that of sporophytes (Borlongan et al., 2019).

1.3.1 Gametophyte growth and maturation

Gametophytes may undergo gametogenesis within approximately one week if conditions are right and produce gametes, or they may remain vegetative and grow to produce filaments of several cells (Lüning and Neushul, 1978; Lüning, 1980; Lüning, 1981; Schiel and Foster, 2006). In order to become fertile, female gametophytes require blue light and this requirement is dependent on temperature – at lower temperatures less blue light is necessary to induce maturation (Lüning and Dring, 1972). Moreover, high temperatures prevent gametogenesis – 20 °C in the case of *S. latissima* (Lüning and Dring, 1972; Lee and Brinkhuis, 1988). Therefore, in laboratory conditions, if only exposed to red light, gametophytes will tend to growth vegetatively, as growth is unaffected by light quality (Lüning and Dring, 1975). The upper temperature tolerance of *S. latissima* gametophytes is 23 °C (Bolton and Lüning, 1982; Lee and Brinkhuis, 1988). In addition, female gametophytes require iron to undergo oogenesis (Motomura and Sakai, 1981; Lewis et al., 2013). Once eggs are released, a pheromone, lamoxirene, is also produced that induces mature male gametophytes to discharge spermatozoids, and subsequently attracts spermatozoids to the egg, facilitating synchronization of the maturation and reproduction process (Lüning and Müller, 1978; Maier et al., 2001).

When the environmental conditions required for fertilization are not met, gametophytes remain vegetative (up to 30 years in laboratory conditions, Druehl et al., 2005; Martins et al., 2019) and this state in the field has been compared to plants' "seed banks". In natural conditions, vegetative gametophytes might resist adverse conditions and then develop once the environment is favorable. Therefore they can replenish populations after a disturbance, such as storm or heat wave; and are essential for the life cycle of annual plants (Schiel and Foster, 2006). Although typically perennial (Bartsch et al., 2008), *S. latissima* has been reported to behave as annual at the southern rear-edge of its distribution in USA, possibly due to die-off during the hot summer (Lee and Brinkhuis, 1986). The persistence of early-life history stages throughout summer ensures a successful recruitment in autumn (Lee and Brinkhuis, 1986).

In laboratory conditions, control of the transitions between the life stages of kelps allows for scientific experimentation and has applications in seaweed aquaculture (Charrier et al., 2017). Namely, a combination of applying high temperatures, removing iron from the nutrient solution added and limiting exposure to blue light (commonly by exposing to red light only) retain the gametophytes in a vegetative state. These stock cultures of gametophytes allow for the preservation of different genotypes until further use or for conservation of genetic resources. In turn, offering the optimal set of conditions induces fertility and allows for sporophyte production at the convenience of the researcher and/or producer

(Scoggan et al., 1989). The establishment of separated male and female gametophyte cultures allows for hybridization experiments with the goal either to understand species boundaries or to develop better cultivars for aquaculture. This practice has been successful in aquaculture facilities in Asia and resulted in the selection of cultivars with the desired characteristics for production (Westermeier et al., 2010; Robinson et al., 2013; Bartsch, 2018). A very important trait to select for is high temperature tolerance that allows for continuous production throughout the summer months and expansion of aquaculture set-ups to warmer waters and it grants sporophytes the ability to withstand current and future warming trends (Pang et al., 2007; Zhang et al., 2011; Kim et al., 2017). Assessing the differential response of the gametophytes gender to temperature is relevant as crossing experiments have indicated that heat sensitivity is transmitted through females (Martins et al., 2019), however further research is necessary on this topic. In addition, sex ratios in kelps have been shown to shift in response to temperature (Izquierdo et al., 2002; Nelson, 2005; Oppliger et al., 2011). Specifically, in *S. latissima* a higher proportion of males compared to female gametophytes was observed at higher temperatures (Lee and Brinkhuis, 1988).

1.4 *The kelp Saccharina latissima*

The systematic classification of the kelp *Saccharina latissima* is as follows:

Empire: Eukaryota
        Kingdom: Chromista
                Infrakingdom: Heterokonta (=Stramenopiles)
                        Phylum: Ochrophyta
                                Class: Phaeophyceae
                                      Order: Laminariales
                                          Family: Laminariaceae
                                                Genus: *Saccharina*
                                                  Species: *Saccharina latissima*
(The UniProt Consortium, 2018; Guiry and Guiry, 2019; Horton et al., 2019)

Saccharina latissima (Figure 3, Lane et al., 2006) has a wide latitudinal range in the Northern hemisphere from polar to temperate waters. In the eastern Atlantic, it can be found from the Arctic (>80°N) until the North of Portugal (41.5°N) and from the upper subtidal until 30 meters depth. The optimum growth range for *S. latissima* is between 10 °C and 15 °C and above 20 °C the mortality rate is high (Fortes and Lüning, 1980; Bolton and Lüning, 1982). Concomitantly, *S. latissima* exhibits optimum growth between absolute salinities (S_A) 23 and 31, a strong reduction at S_A 16 and a high mortality < S_A 8 (Gerard et al., 1986). Nevertheless, *Saccharina latissima* is present in the Baltic Sea where salinity reaches around S_A 10 at the South-west Baltic proper (Kautsky and Kautsky, 2000). The alga displays a remarkable dynamic acclimation capability (Bischof et al., 1998). Nevertheless, declines in its distribution and biomass are increasingly reported (Sweden: Eriksson et al., 2002; Germany: Pehlke and Bartsch, 2008; Norway: Moy and Christie, 2012; Canada: Filbee-Dexter et al., 2016; USA: Witman and Lamb, 2018; Portugal and Spain: Casado-Amezúa et al., 2019) and are especially concerning when declines lead to a shift to ephemeral turf algae with disproportionate changes in the coastal communities (Eriksson et al., 2002; Filbee-Dexter and Wernberg, 2018). Furthermore, niche modelling predicts a northward shift of the

distributional range of *S. latissima* between 138 - 552 kilometers in Europe (Westmeijer et al., 2019) and 0 - 203 km in Northwestern Atlantic (Wilson et al., 2019) by 2100 depending on the climate change scenario being considered. Therefore, range contractions at the southern and central range will be paralleled with expansion into the Arctic as the ice cover retreats (Müller et al., 2009; Assis et al., 2018). Moreover, the occurrence and abundance of the species over large stretches of the European coast remains unknown (Araújo et al., 2016), which hinders our understanding of its conservation status.

Figure 3 The sugar kelp *Saccharina latissima*. The species can be identified by an undivided frond with distinct bullations (= depressions) and a ruffled side. It has a claw-like holdfast and a short and flexible stipe (http://www.seaweed.ie/descriptions/ Saccharina_latissima.php). Left side: picture of *S. latissima* (center) at low tide in Roscoff, Brittany. Photo by Cátia Monteiro; right side: illustration of *S. latissima* by Harvey (1846)

1.5 Responses to the environment

1.5.1 Responses of seaweeds to climate change

Climate change is significantly impacting the structure and function of marine ecosystems worldwide (Parmesan and Yohe, 2003; Harley et al., 2006). Continuous increase in temperature will result in changes in species distribution ranges (Lima et al., 2007; Hawkins et al., 2009; Poloczanska et al., 2014). Higher temperatures drive species close to their thermal tolerance limits at the southern distribution range reducing their fitness and in some cases resulting in local extinction (Hampe and Petit, 2005). At the same time, warming allows species to move northwards as well as to improve productivity in areas where they have been living under sub-optimal temperatures, namely the Arctic (Bartsch et al., 2016). Species survival and success in this altered environment will depend on both biotic interactions and abiotic factors (Müller et al., 2009). Reports on the impacts of global warming on seaweeds are mounting (e.g. Moy and Christie, 2012; Andersen et al., 2013; Mineur et al., 2015; Bartsch et al., 2016; Thomsen et al., 2019). As sessile organisms they cannot escape local environmental stress, therefore they need to acclimate to the new conditions or perish (Häder and Figueroa, 1997). Many studies have focused on the response of seaweeds to single stress factors, however this approach holds limited ecological relevance as species are subjected to combined stress factors in the field (Mineur et al., 2015). The result may not simply be the addition of each single stress effect, but have a synergistic or antagonistic effect (Lotze and Worm, 2002). Moreover cross-acclimation may occur, where the acclimation to

one stressor improves the tolerance of the algae to a second, different stressor (Leshem and Kuiper, 1996). Although described in plants, the prevalence of this phenomenon in seaweeds has only been investigated once, a study indicating that acclimation to low salinity in the kelp *Alaria esculenta* increased the tolerance to UV stress (Springer et al., 2017). However, information on these interactions is still scarce. Some authors have shown the interactive effects of temperature and UV radiation on macroalgae and the single effects of these two factors have been extensively studied (e.g.Bischof et al., 1998; Aguilera et al., 2002; Holzinger et al., 2011; Parages et al., 2013).

Salinity is expected to change in coastal systems in the future under current climate change scenarios due to increased precipitation, increased wind speed and ice melting in polar environments, with likely significant impacts on the communities (Manabe and Stouffer, 1995; Wiencke et al., 2007; Holt et al., 2010; Johannesson et al., 2011; Alexander et al., 2013; Vuorinen et al., 2015). Nonetheless, salinity is remarkably underrepresented in physiological studies despite its relevance to performance. Hypo- and hypersalinity require osmotic adjustment by the algae. First, water fluxes rapidly follow the osmotic gradient resulting in changes in cell turgor pressure and cell volume. This is followed shortly by changes in ionic balance (up to few hours) and later by changes in concentration of osmolytes (up to three days) (Kirst, 1990). A known osmolyte in brown algae is mannitol, that is also a major photosynthetic product and carbon storage compound (Iwamoto and Shiraiwa, 2005; Barboza et al., 2019). As a result of exposure to salinity gradients, changes in phenolics, antioxidant enzymes, phycobiliproteins, and fatty acids in seaweeds were also reported (Kumar et al., 2010; Connan and Stengel, 2011; Stengel et al., 2011). Furthermore, salinity variation also impacts photosynthetic efficiency (Barboza et al., 2019) by processes such as inhibition of electron flow on the oxidizing side of photosystem II (Satoh et al., 1983), pigment content (Schubert et al., 1993), and stimulation of cyclic electron transport at photosystem I (Endo et al., 1995).

Impacts of climate change, however, are not equally distributed across the globe. The Arctic is warming faster than any other region and the resulting ice melting is inducing increases in freshwater input, changes in light regimes, sedimentation and carbon cycling in near-shore habitats. Therefore, changes in abundance and biomass of macroalgae species can be expected (Filbee-Dexter et al., 2018). The role of kelp in this boreal ecosystem is especially relevant as they persist through the dark winter unlike other primary producers, therefore continuously supporting the food webs during this period (Berge et al., 2015). Kelps have developed adaptations to the polar environment by fine-tuning their phenology (="the seasonal timing of species' life-cycle events") with the strong seasonal patterns experienced there (Wiencke et al., 2007; Wiencke et al., 2009). Shifts in phenology have been recorded in response to climate change. These shifts might result in mismatch between development and food availability and between different levels of the trophic web (Parmesan and Yohe, 2003; Poloczanska et al., 2013). A recent review identified shifts in the timing of reproduction in temperate macroalgae driven by climate warming, revealing yet another impact of climate change on seaweeds (de Bettignies et al., 2018).

In certain locations, in addition to climate change, pollution, biological invasions and habitat degradation amongst others are also threatening seaweed ecosystems. Therefore, it is of prime importance to consider all these anthropogenically driven impacts in conservation efforts (Moy and Christie, 2012; Araújo et al., 2016; Small, 2018; Wernberg et al., 2019).

1.5.2   Local adaptation and phenotypic plasticity

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Definitions

Rear edge = **trailing edge** = **low-latitude limit** = populations at the low latitude limit of species distribution. In case of the northern hemisphere, it corresponds to the southernmost edge. (Hampe and Petit, 2005)

Expanding edge = **leading edge** = **high-latitude limit** = populations at the high latitude limit of species distribution. In case of the northern hemisphere, it corresponds to the northernmost edge. (Hampe and Petit, 2005)

Peripheral populations = **range margins** = populations at both limits of the distributional range of a species. (Gibson et al., 2009)

Fitness = the ability of an organisms to survive and reproduce. (Holderegger et al., 2006)

Phenotype = "Phenotype refers to the outward appearance of a genotype; it is the outcome of the interaction between genotype and environment." (Holderegger et al., 2006)

Phenotypic plasticity = "the range of phenotypes a single genotype can express as a function of its environment." (Nicotra et al., 2010)

Ecotype = "locally adapted populations that are phenotypically and genetically differentiated for adaptive traits." Conner and Hartl (2004)

Acclimation = "short-term physiological adjustments that occur during a lifetime in response to transitory changes in environmental conditions." (Morgan-Kiss et al., 2006) Acclimation is the underlying mechanism resulting in phenotypic plasticity.

Adaptation = "the process of genetic change that accumulates over a time scale of many generations in response to an organism's specific environmental niche." (Morgan-Kiss et al., 2006)

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Species may respond to environmental change through phenotypic plasticity or by adaptation (for a list of relevant definitions please see above). Phenotypic plasticity takes place when changes in fitness in result of environmental variation are not genetically determined and the species is able to tolerate changes by temporarily adjusting its physiological mechanisms. Local adaptation occurs when there are genetically fixed differences between populations that result in the adjustment of optimal physiological ranges (fitness) to the conditions experienced locally. If there is evidence for adaptation of certain populations they can be defined as ecotypes (Conner and Hartl, 2004; Nicotra et al., 2010). An ecotype will perform better at the local conditions than another ecotype from a distant population and a given trait will be expressed irrespective of environmental variation (Kawecki and Ebert, 2004). These two different mechanisms (phenotypic plasticity and adaptation) play a role in establishing species distribution. Phenotypic plasticity happens in short-time scales and allows the species to respond quickly to environmental change, as opposed to adaptation that acts on long-term scales. Current rates of climate change might surpass species ability to adapt and therefore acclimation through phenotypic plasticity might be the key to allow for species' persistence (Valladares et al., 2014). For adaptation to occur a selective force has to act on a population for sufficiently long period of time and it has to be stronger than the genetic homogenization driven by gene flow (Nicotra et al., 2010). Gene flow itself depends on the dispersal capacity of the species (i.e. the ability of individuals to move between populations) and on disper-

sal barriers. Dispersal capacity of kelps is restricted and considerably lower than for other marine organisms. Moreover, dispersal barriers such as lack of rocky substrate are common occurrences (Valero et al., 2011; Durrant et al., 2014). Thus, significant population differentiation is common among kelps and we can expect local adaptation to occur frequently for these brown algae (King et al., 2018). Indeed, a recent review revealed that 90% of marine macrophytes (= seagrasses and macroalgae) present population-specific responses to high temperatures (King et al., 2018). If locally adapted to the high temperatures routinely experienced, populations at the southern edge might be more resilient than expected, and the opposite might hold true for central and northern populations. Therefore, deciphering which mechanism is at play for a certain species will inform species distribution models and conservation measures.

1.5.3 Ecotypic differentiation in *S. latissima*

Ecotypic differentiation has been reported between several populations of *S. latissima* in the Northeast- (Müller et al., 2008; Olischläger et al., 2014; Olischläger et al., 2017) and Northwest Atlantic (Gerard et al., 1986; Gerard and Du Bois, 1988; Gerard, 1988; Gerard, 1990). Furthermore, recent genetic data further reveals the process of local adaptation in the North-east distribution of *S. latissima* to an extent that speciation might be in progress. The six populations tested by Guzinski et al. (2016) with microsatellite markers (three French populations – southern Brittany, Northern Brittany; Denmark; Sweden; Spitsbergen, Norway) were significantly genetically differentiated. In addition, absence of admixture between French and Norwegian populations was reported and within-population genetic diversity was low. Neiva et al. (2018) identified two phylogroups in Europe: a northern phylogroup constituted by Spitsbergen and Bergen in Norway, Greenland, Russia and Iceland; and a southern phylogroup (Brittany, France and northwest Spain) based both on mitochondrial DNA and microsatellite data. Furthermore, a study in a smaller geographical scale comparing brackish (Denmark) and marine (Norway and Sweden) populations of *S. latissima* revealed low genetic diversity within the brackish population and significant differences between brackish and marine populations (Møller Nielsen et al., 2016). Moreover, differentiation at the physiological level is already evident – the brackish Aarhus bay population of *S. latissima* showed a lower production when compared to other populations at the same latitude and authors suggested that low salinity might be a contributing factor together with high summer temperatures (Nielsen et al., 2014).

In the northwestern Atlantic, thermal ecotypes have been identified for algae from the populations of Maine (northern population) and New York (southern limit distribution) in laboratory common garden experiments with field sporophytes (Gerard et al., 1986; Gerard and Du Bois, 1988) and in field measurements (Gerard and Du Bois, 1988). Specimens from the southern limit distribution that experience higher summer temperatures than the northern ones survived temperatures above 20 °C, while algae from the northern distribution did not. Moreover, differences were only apparent at high temperatures, while at lower temperatures growth and survival patterns were similar (Gerard and Du Bois, 1988). Within the same populations, ecotypic differentiation was uncovered between individuals from shallow, deep and turbid environment in their light response characteristics, namely photosynthetic parameters, carbon assimilation and growth (Gerard, 1988). Further work targeting the same shallow, deep and tur-

bid populations further corroborates ecotypic differentiation for these populations, this time for both sporophytes and gametophytes cultivated from spores in laboratory. Differences between populations across parameters tested were not always consistent for both gametophytes and sporophytes which revealed phase-specific adaptation (Gerard, 1990). In Alaska, USA, Spurkland and Iken (2011) did not detect ecotypes between glacially influenced and oceanic sites with contrasting irradiance and salinity levels in three-weeks laboratory experiments. However, in a follow-up study with *in situ* reciprocated transplants, seasonal growth patterns reflected the environment of origin irrespective of growth site and therefore authors suggested a genetic basis for these differences (Spurkland and Iken, 2012).

The variability described above in phenotypic plasticity and ecotype formation in *S. latissima* is based partially in different approaches (laboratory experiments, reciprocal transplants, *in situ* measurements), in different factors being targeted (temperature, irradiance, salinity) and also different tested parameters (growth, survival, fitness parameters: *F*v/*F*m, biochemical composition). These differences make it difficult to systematically compare these results and warrant discussion on what is the most sensible parameter to assess phenotypic plasticity/local adaptation. Field reciprocal transplants of specimens originating from two distinct populations are often used to test for local adaptation (Kawecki and Ebert, 2004), however in the case of *S. latissima* reciprocal transplants cannot be applied in Spitsbergen because the area is protected and sporophytes originated in other locations cannot be brought there (Svalbard Environmental Protection Act, 2001). Moreover, considering the high genetic differentiation between populations of *S. latissima*, concerns with genetic contamination are warranted (Guzinski et al., 2016; Luttikhuizen et al., 2018). Therefore, a common garden experiment is the ideal experimental design to assess local adaptation by exposing individuals from different populations to the same environmental conditions (Kawecki and Ebert, 2004).

Overall, previous studies revealed that *S. latissima* has adapted to local conditions throughout its wide distributional range and therefore responses to stressors are site-specific and cannot be extrapolated from one population to the whole species complex. Consequently, efforts must be made to integrate data from as many populations as possible as environmental parameters at the local scale should be integrated in any modelling exercise (Bennett et al., 2015). Moreover, the selection pressures underlying this evolutionary adaptation are not well understood and understanding the future of the species under current environmental change is dependent on more knowledge on the topic.

1.6   Transcriptomic responses to abiotic stress

A widely used method to assess the genetic basis of stress response is transcriptomics. Transcriptomics (or gene expression) measures the abundance of mRNA in a tissue thereby providing expression levels of genes following a stimulus (Wang et al., 2009). Transcriptomic data has been mounting, especially for model-species such as the plant *Arabidopsis thaliana* (Honys and Twell, 2004; Gong et al., 2005; Rest et al., 2016; Zhang et al., 2017). In contrast, transcriptomics approaches in brown algae are still scarce and knowledge of their acclimation mechanisms to the changing conditions experienced in coastal environments can benefit from the application of RNA-sequencing. Previous work in the Laminariales *S. latissima* (Heinrich et al., 2012b; Heinrich et al., 2015; 2016), *Laminaria digitata* (Roeder et al., 2005; Ritter et al., 2008) and *Saccharina japonica* (Liu et al., 2014) as well as other brown seaweeds, such as *Ectocarpus*

(Dittami et al., 2009; Ritter et al., 2014) and *Fucus* (Pearson et al., 2010), revealed large reprogramming of gene expression by abiotic stress and provided the foundation for the research undertaken during this PhD. While survival, reproductive success and growth are obviously relevant parameters that integrate all the metabolic processes taking place in the algae they lack insights into the underlying molecular mechanism of acclimation or adaptation. Moreover, while survival and growth might be maintained under contrasting conditions this might come at a cost for reproduction as energy might be deviated from reproduction to survival strategies. Therefore gene expression offers an opportunity to assess metabolic reorganization taking place after a stress exposure even before there is an obvious signal in physiological performance (Heinrich et al., 2015). Furthermore, although local adaptation is intensively studied, the molecular mechanisms underlying this process remain poorly understood, especially in non-model species (Savolainen et al., 2013; Kenkel and Matz, 2016; Avia et al., 2017). Transcriptomics might help identifying the biological processes involved in adaptation. Moreover, it can contribute to the identification of populations relevant for conservation before they are seriously affected by ongoing anthropogenic changes. In the seagrass *Zostera marina*, Franssen et al. (2011) reported diverging gene expression profiles during recovery after experimental heat-wave between northern and southern populations, but not during heat-wave. Specimens from the north population failed to recover from the heat stress and up-regulated genes related to protein degradation. In the coral *Porites astreoides*, Kenkel and Matz (2016) described clear differentiation between gene expression profiles between inshore and offshore populations. Moreover, by transcriptomic analysis of transplanted corals they identified high gene expression plasticity in corals originated from the more variable environment, inshore, than the more stable one, offshore. Therefore, transcriptomic analysis can elucidate biological mechanisms diverging between populations and underlying differential response to stress.

1.7 Aim

The study presented here aims to understand the responses of *Saccharina latissima* in the Atlantic to multiple stressors. By including the largely overlooked life stage of gametophytes, we expect to significantly contribute to the understanding of adaptive mechanisms defining distribution and success of the kelp *S. latissima*. By investigating populations covering the distribution range (Arctic, North Sea, North Atlantic) we expect to achieve a holistic understanding of the mechanistic base of abiotic stress tolerance of the species and the metabolic pathways supporting its high acclimation potential. These results will provide valuable information to predict shifts in the species' distribution patterns in the future due to global warming.

1.8 Research questions and hypotheses

1. Does acclimation to temperature in sporophytes of *S. latissima* change tolerance towards salinity stress?

Saccharina latissima specimens from Roscoff, France and Spitsbergen, Arctic were subjected to a hyposalinity treatment after temperature acclimation (0 °C, 8 °C and 15 °C). We hypothesized that temperature and salinity stress would have interactive effects on algal performance. We further hypothesized that algae cultured at 8 °C would perform better when subjected to salinity stress than the ones cultured at 0 °C and at 15 °C. An increase in temperature increases overall metabolic activity but when it reaches close to the upper tolerance limit stress responses might be too costly as synchronous activation of different stress pathways is not probable beyond a certain stress level.

2. Do sporophytes of the populations from the Northern (Arctic) and Central (Brittany) distributional range present the same transcriptomics and/ or physiological responses to stress?

We hypothesized that populations from Brittany would perform better under high temperatures than populations from the North and vice-versa. We also expected to find differences in the metabolic pathways expressed between the two populations, such as in the transcripts responsible for reactive oxygen species (ROS) scavenging mechanism and photosynthetic components that would be indicative of the strategies of adaptation to both low and high temperature.

3. Is there a sex-biased gene expression in the vegetative gametophytes of *S. latissima*?

Vegetative male and female gametophytes originated from spores collected in Helgoland, German Bight, North Sea were exposed in a laboratory experiment to a high temperature of 20 °C and a low temperature of 4 °C after a period of cultivation at 12 °C. We hypothesized that comparison of gene expression profiles between male and female gametophytes would reveal differences that can be connected with the sexual dimorphism and differences in physiology that anticipate sexual maturity.

4. Is there an interaction between temperature and sex effects in vegetative gametophytes of *S. latissima* at the transcriptomic level?

Based on the same laboratory experiment described in research Question 3, we addressed the question of how the vegetative gametophytes respond to changes in temperature and how sexes might respond differently to them. Previous studies demonstrated a differential response of male and female kelp gametophytes to temperatures at the physiological level. Therefore we hypothesized that we can identify differences at the gene expression profiles as well, namely in stress response related pathways.

1.9 Thesis outline

Saccharina latissima faces environmental variation throughout its distributional range. Increasing temperatures are experienced by *S. latissima* from the northern to the southern distribution, with relevant local anomalies (e.g. Helgoland). Moreover, large-scale salinity patterns are also observed – the Baltic sea is a brackish sea and the North Atlantic is a fully marine environment. Also regarding salinity, relevant small-scale variation occurs after precipitation events and freshwater input from land that might lead to local reduction in salinity e.g. Arctic fjords. For the species to be able to survive and succeed in these fluctuating environments, acclimation mechanisms are necessary and those have been studied extensively at both physiological and biochemical level. However, the genetic basis underlying these acclimation mechanisms remains poorly understood. Furthermore, a considerable knowledge gap is observed for the microscopic life stages, for which the abiotic stress impacts are less understood. Differences in stress susceptibility between gametophytes and sporophytes have been documented warranting further investigation of specific life-stage responses. Moreover, it is during the microscopic life stages that sexual reproduction takes place and therefore understanding abiotic stressors impact on reproductive success is of paramount importance considering the mounting evidence of the relevance of this phase for species success.

To assess the effect of geographical variation in *S. latissima*, sporophytes were raised from spores collected in Roscoff (Brittany, North Atlantic, at the geographical centre of distribution) and spores collected in Spitsbergen (Svalbard, Norway, Arctic, at the northern limit of distribution) under the same conditions in the laboratory (common garden). At the juvenile stage (after three months of cultivation, 5-10 cm length), sporophytes were exposed to the same experimental design by location. Several physiological measurements and RNA extractions were performed for sporophytes from both locations at different time points. Considering the extent of the data gathered and in order to better address the research Questions, this data was tackled in three different Chapters (3, 4 and 5). The respective experimental design is presented in Figure 4.

In Chapter 3, I assess responses of *S. latissima* from the Arctic to temperature and salinity variation at both physiological and transcriptomic level and target research Question 1 defined above. These responses are discussed in the context of the current warming rates in the Arctic. In Chapter 4, a comparative analysis between the two geographical sites is provided solely at the transcriptomic level targeting

research Question 2. I discuss similarities and disparities in the gene expression profiles of algae from Roscoff and Spitsbergen and relate this to the environmental parameters of the original sites. Then, I explore how these responses might correlate with phenotypic plasticity and/or ecotypic differentiation in *S. latissima* at the transcriptomic level (chapter 4) and physiological level (chapter 5). In Chapter 5, physiological data obtained throughout the temperature and salinity experiment in the sporophytes from Roscoff is analysed. The acclimation mechanisms involved and how this relates to the physiological tolerance of the species to temperature and salinity variation is discussed. This Chapter further addresses research Questions 1 and 2. Then, I performed a second laboratory experiment targeting gametophytes, the microscopic, haploid life-history stage (Chapter 6). Considering the limited information available for this life stage, the experiment was focused on a single abiotic factor as interactions might prove too difficult to interpret. Gene expression profiles changes driven by temperature (4 °C, 12 °C and 20 °C) and sex (male and female) were evaluated (experimental design displayed on Figure 5). Temperature levels applied were chosen to mirror temperature range experience at the site of origin: Helgoland, German Bight, North Sea.
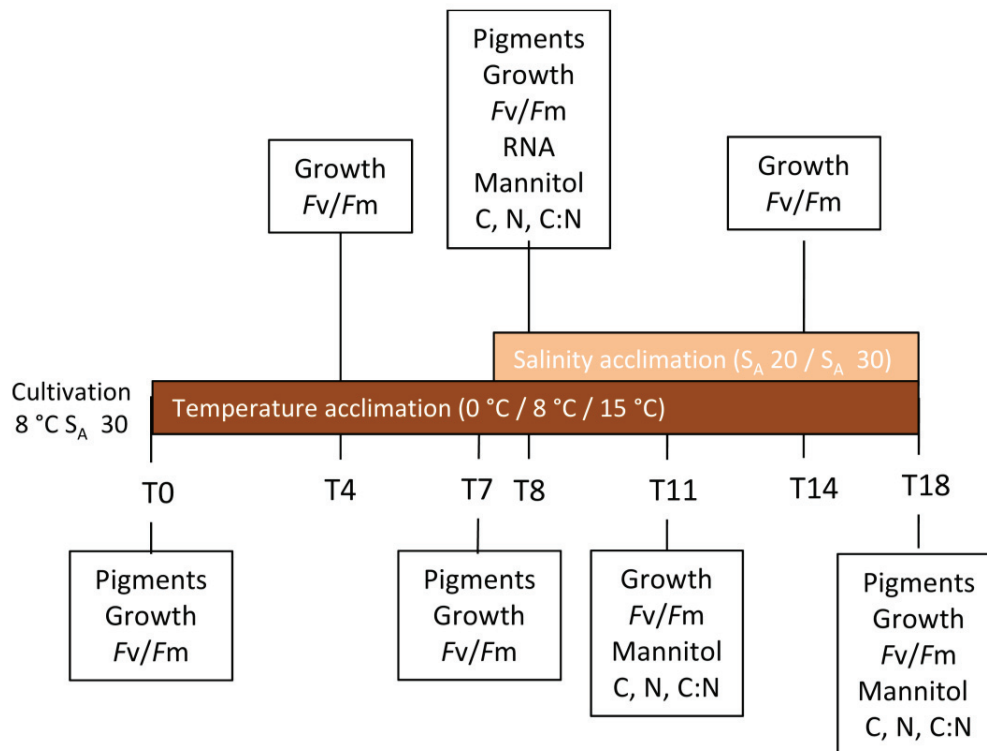
Figure 4 Experimental design and parameters measured during the temperature and salinity acclimation experiment in sporophytes of *Saccharina latissima* (Chapters 3, 4 and 5). In laboratory, sporophytes from Roscoff, North Atlantic and from Spitsbergen, Arctic were raised under the same conditions (cultivation 8 °C S_A 30). At the start of the experiment, they were acclimated for seven days at 0 °C, 8 °C and 15 °C. After seven days, they were exposed to a low salinity of S_A 20 for eleven days, in a total of eighteen days of experiment. Several parameters were measured at relevant time points during the experiment. Pigment content, growth, maximal quantum yield of photosystem II (*Fv/Fm*), mannitol content, carbon (C), nitrogen (N) and carbon to nitrogen ratio (C:N) and gene expression profiles after RNA-sequencing (RNA).
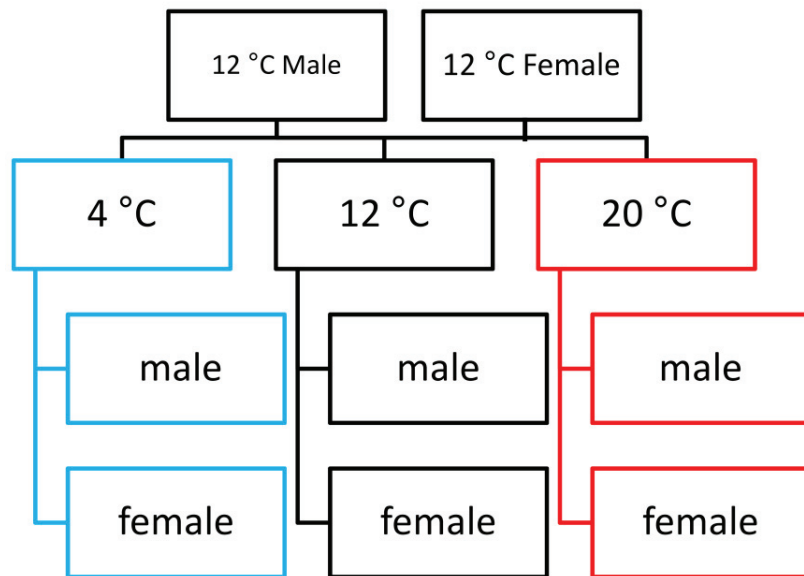
Figure 5 Experimental design applied during the temperature acclimation experiment in gametophytes of *Saccharina latissima* by sex. Gametophytes cultures were grown at 12 °C with sexes separated. At the start of the experiment, male and female gametophytes were exposed to 4 °C, 12 °C and 20 °C for fourteen days. Growth and *F*v/*F*m were measured at the start and at the end of the experiment. Samples for RNA extraction were taken at the end of the experiment.

1.10 List of publications and declaration of contributions

Publication 1: Responses of the kelp *Saccharina latissima* (Phaeophyceae) to the warming Arctic: from physiology to transcriptomics

Authors: Huiru Li, Cátia Monteiro, Sandra Heinrich, Inka Bartsch, Klaus Valentin, Lars Harms, Gernot Glöckner, Erwan Corre and Kai Bischof

Journal: *Physiologia plantarum*, doi:10.1111/ppl.13009

Contribution of the candidate in % of the total workload:
Experimental concept and design: 40%
Experimental work and acquisition of the data: 40%
Data analysis and interpretation: 70%
Preparation of figures and tables: 5%
Drafting of the manuscript: 60%

Publication 2: Is geographical variation driving the transcriptomic responses to multiple stressors in the kelp *Saccharina latissima*?

Authors: Cátia Monteiro, Huiru Li, Kai Bischof, Inka Bartsch, Klaus Ulrich Valentin, Erwan Corre, Jonas Collén, Lars Harms, Gernot Glöckner and Sandra Heinrich

Journal: BMC Plant Biology, https://doi.org/10.1186/s12870-019-2124-0

Contribution of the candidate in % of the total workload:
Experimental concept and design: 70%
Experimental work and acquisition of the data: 60%
Data analysis and interpretation: 70%
Preparation of figures and tables: 100%
Drafting of the manuscript: 95%

Publication 3: Temperature dependent sex-biased gene expression in the gametophytes of the kelp *Saccharina latissima*

Authors: Cátia Monteiro, Sandra Heinrich, Inka Bartsch, Klaus Valentin, Erwan Corre, Jonas Collén, Lars Harms, Gernot Glöckner and Kai Bischof

Journal: Frontiers in Marine Science, doi: 10.3389/fmars.2019.00769

Contribution of the candidate in % of the total workload:
Experimental concept and design: 70%
Experimental work and acquisition of the data: 100%
Data analysis and interpretation: 70%
Preparation of figures and tables: 100%
Drafting of the manuscript: 95%

Publication 4: Physiological responses to temperature and salinity variation in sporophytes of the kelp *Saccharina latissima* from Roscoff, France

Authors: Cátia Monteiro, Huiru Li, Nora Diehl, Inka Bartsch, Sandra Heinrich, Kai Bischof and Jonas Collén

Journal: to be defined; *in preparation*

Contribution of the candidate in % of the total workload:
Experimental concept and design: 70%
Experimental work and acquisition of the data: 60%
Data analysis and interpretation: 70%
Preparation of figures and tables: 100%
Drafting of the manuscript: 95%

2 Methodological aspects

To investigate acclimation mechanisms to abiotic stress in *Saccharina latissima* I explored both physiological parameters and transcriptomic analysis in a set of laboratory experiments. Physiological and biochemical parameters included chlorophyll fluorescence measurements (*F*v/*F*m), growth and content of pigments, mannitol, carbon and nitrogen. The respective analytical procedures have been readily established previously, and methodological details can be found in Chapter 3 (growth, chlorophyll fluorescence measurements (*F*v/*F*m), pigments) and Chapter 5 (growth, chlorophyll fluorescence measurements (*F*v/*F*m), pigments, mannitol, carbon and nitrogen). For transcriptomic analysis, however, novel non-standardized approaches had to be developed. In this respect, the main challenge of the project was the in-depth analysis of a non-model species, for which only fragmented genomic information is at hand. Furthermore, due to some peculiar biochemical characteristics in kelp (i.e. cellular phlorotannin and high carbohydrate loads) even well-established extraction methods had to be optimized *de novo* and tailored to the system under investigation. Therefore, an overview of the methodology and workflow applied to the transcriptomic data resulting from RNA-sequencing from *S. latissima* is provided below.

2.1 RNA extraction and sequencing

Extracting good quality RNA from Phaeophyta is challenging due to the high content of polysaccharides and phenolic compounds (Pearson et al., 2006; Heinrich, 2012). We followed the protocol by Heinrich et al. (2012a) that has proved successful in *S. latissima* sporophytes. This thesis confirms that this protocol enables good quality RNA extraction for both sporophytes and gametophytes of the species. It is important to note that this protocol is time-consuming (four hours per four samples), involves working with liquid nitrogen and requires extractions to be performed within four weeks for optimal RNA quality. After extraction, RNA quality was analysed by the NanoDrop ND-1000 UV-Vis Spectrophotometer and Agilent 2100 Bioanalyzer (Agilent Technologies, Germany). For sequencing at Cologne Center for genomics (CCG), cDNA libraries were prepared with an Illumina TruSeq RNA Library Prep Kit according to the manufacturer protocol. The libraries were sequenced on an Illumina Hiseq 2500 and 75 bp paired reads were clipped using default values of the Illumina software.

2.2 Bioinformatics analysis of RNA-sequencing

Previous transcriptomic studies in *S. latissima* used microarrays (Heinrich et al., 2012a; Heinrich et al., 2012b; Heinrich et al., 2015; 2016). In this study, instead we used RNA-sequencing to measure expression of genes in the experiments performed as this offers several advantages over microarrays (Wang et al., 2009). Namely, microarrays are dependent on existing knowledge of genome sequences; they produce a high background noise and are unable to detect lowly expressed genes (Wang et al., 2009). Hence, since RNA-sequencing is a novel technology that only recently became financially feasible and therefore accessible to non-model organisms, this thesis is to our knowledge the first to include transcriptomic analysis using RNA-sequencing following a (biotic or abiotic) stimulus in *S. latissima* and contributes to a still limited pool of RNA-sequencing studies in seaweeds. Raw reads resulting from the se-

quencing undertaken during this thesis are available on the Array express repository under the accession numbers E-MTAB-8267 for the gametophyte data and E-MTAB-7348 for the sporophyte data and therefore are valuable genomic resources that can be further analysed with several applications. For example, this data is currently being used to improve the annotation of the genome of *S. latissima* under the project Phaeoexplorer. Phaeoexplorer aims to generate a genomic data resource constituted of complete, annotated genome sequences for 69 strains corresponding to 47 species of brown algae (representing all major orders) and four unicellular and multicellular sister species.

As there is no available genome yet for the species studied, we performed a *de novo* transcriptome assembly. In order to obtain the best remapping rates of the reads for downstream analysis, we assembled two transcriptomes, one based on the experiment with sporophytes in response to salinity and temperature variation and a second one based on the experiment with gametophytes in response to temperature.

The bioinformatics pipeline followed was similar for both *de novo* transcriptomes (see summary in Figure 6). For further details, see Material and Methods in Chapters 3, 4 and 6.
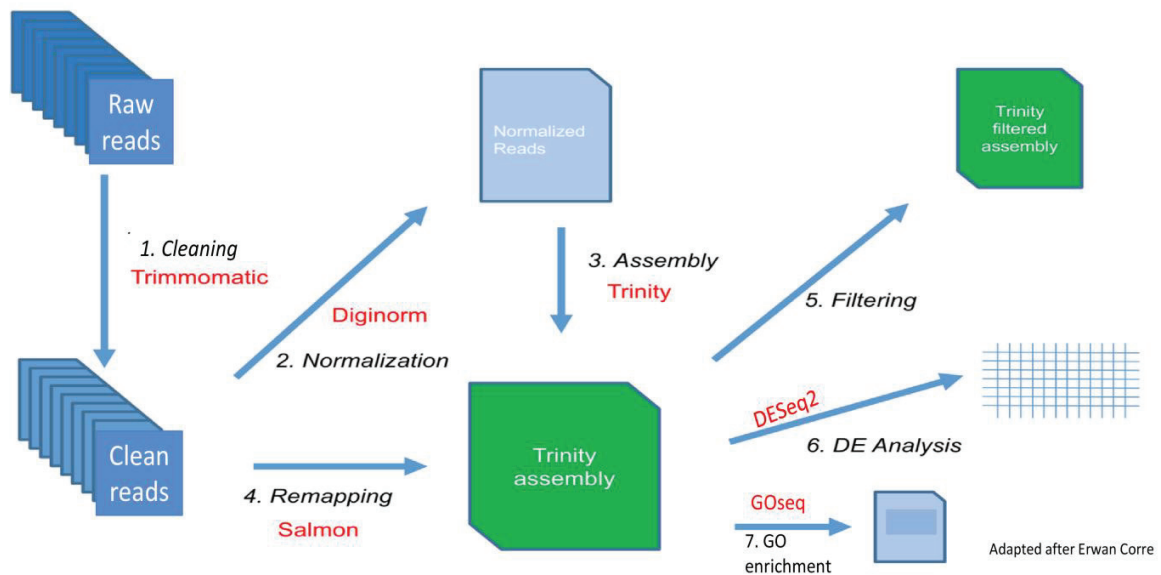
Figure 6 Bioinformatics pipeline followed for the RNA-seq data obtained in this thesis. In numbers, the sequential steps since obtaining the DNA sequences until statistical analysis. First, raw reads were filtered according to defined quality parameters (1), the clean reads were normalized (2) before the *de novo* assembly (3). After filtering this assembly, statistical analysis was performed on the transcriptome. First, differential expression was analyzed (6), followed by GO enrichment (7). In red, the software used for each step.

2.3 Interpretation and visualization of transcriptomic data

2.3.1 Functional annotation

An important step in analysis of transcriptomic data is the annotation of expressed genes. Considerable efforts have been made to improve annotation rates and several databases and tools are now accessible (e.g. Buchfink et al., 2015; Bryant et al., 2017; Huerta-Cepas et al., 2017; The Gene Ontology Consortium, 2018). Annotation is mostly based on the search for orthologues, under the assumption that genes with similar sequences in other taxa will have similar functions as the taxa being studied. This assumption has been verified and enables poorly studied groups such as brown algae to benefit from functional information gathered by studies on model species (Gabaldón and Koonin, 2013). However, high annotation rates are hampered by the evolutionary distance between brown algae and plants, green and red algae (Cock et al., 2010) and the unique features that brown algae have developed in response to their environment (Barre et al., 2010; Michel et al., 2010a; b). Moreover, genetic transformation in brown algae has not been accomplished so far which hinders functional analysis in the group (Mikami, 2014). Hence, the function of a large proportion of identified expressed genes in this thesis as in other contemporary work remains unknown (e.g. Heinrich et al., 2012a; Salavarría et al., 2018). Therefore, interpretation of the data has to be performed in light of what is known in better-studied organisms and extrapolations need to be done cautiously. Nevertheless, the high coverage offered by current sequencing technologies means that several hundred genes per treatments have known functions that provide important insight into acclimation mechanisms in brown algae.

2.3.2 Gene Ontology analysis

The Gene Ontology (GO) project provides a standardized vocabulary of the role of gene products and proteins that enables comparisons across organisms. Gene Ontology is divided in three independent domains: Biological process, Molecular function and Cellular component that comprise different aspects of a gene product. Biological process refers to "biological objective to which the gene or gene product contributes", Molecular function to "the biochemical activity (including specific binding to ligands or structures) of a gene product" and Cellular component refers to "the place in the cell where a gene product is active" (Ashburner et al., 2000). A single gene or protein is expected to have at least one GO term within each ontology but it can have several dependent on the knowledge available. As an example, to the protein "Heat shock 70 kDa protein 7, chloroplastic", gene "HSP70-7" were attributed 9 GO terms within Biological process (e.g. GO:0009408, response to heat), 9 GO terms within Molecular function (e.g. GO:0005515, protein binding) and 7 within Cellular component (e.g. GO:0009579, thylakoid) (https://www.uniprot.org/uniprot/Q9LTX9). Moreover, the specificity of a term varies; and more general processes are connected to more specific ones in a parent-child relationship within each ontology. For example, the GO term "response to heat" is a child (more specific) term of the term "response to stress" that in turn is a child term of "response to stimulus" and "response to stimulus" is a child-term of the ontology "Biological process" (Figure 7). Moreover, a GO term can have several parent-terms such as "response to heat" is a child-term of both "response to temperature stimulus" and "response to stress".

Hence, interpreting biological meaning from GO terms can be challenging considering that GO terms can be at the same rank, meaning same depth within the graph, but have different levels of specificity. In addition, there is considerable redundancy between parent-child relationships that further complicate interpretation. Moreover, GO terms are assigned based on available literature and therefore are biased towards the most well-studied species, namely human and mouse. Several programs have been developed to help summarize and interpret the results of GO enrichment analysis in a meaningful way. Web-based approaches such as cateGOrizer can be used to summarize long lists of GO terms. This approach reduces the list of GO terms by categorizing and grouping them into more general terms according to the user's interest or based on available classification methods (Hu et al., 2008). An example is given on the table 1, from a list of 20 GO terms; cateGOrizer provides a list of 12 parent GO terms that can be more easily interpreted. The counts column tells us how many of our input GO terms can be categorized within each given GO term. We believe that this approach is useful when genome-wide analysis provides a long list of significant GO terms particularly to reduce human-biased functions in non-model species. Therefore, this approach was used on Chapters 3, 4 and 6.

QuickGO - https://www.ebi.ac.uk/QuickGO

Figure 7 Ancestor chart of the GO term "response to heat" revealing relationships between GO terms and their hierarchy within the Ontology "Biological process". Specificity of the process increases from parent to child terms, from top to bottom respectively. Figure was obtained from https://www.ebi.ac.uk/ QuickGO/term/GO:0009408

Based on the differentially expressed genes with assigned GO terms, a GO enrichment analysis was performed (Young et al., 2010). This analysis reveals which GO terms are enriched (over-represented) under a certain condition (e.g. higher temperature) compared to others (e.g. control temperature). Hence, enriched GO terms under high temperature indicate the cellular processes that are induced in the algae when exposed to heat. This analysis is often easier to interpret than differential expression; however it is restricted to the genes with GO annotation.

Another useful annotation tool is KOG (Eukaryotic Orthologous Groups) (Tatusov et al., 2003). KOG categories used to prepare the Figure 8 on Chapter 8.1.1. were obtained through the annotation tool egg-NOG-mapper (Huerta-Cepas et al., 2017).

Table 1 Example of an output list from cateGOrizer (Hu et al., 2008) when the classification method "GO slim" was applied on the right, after a given input list of GO terms (on the left).

Input (list of GO terms)		Output of cateGOrizer			
		GO Class ID	**molecular_function**	**Counts**	**Fractions**
GO:0043167	ion binding	GO:0003674	binding	13	23.64%
GO:0045335	phagocytic vesicle	GO:0005488	**cellular_component**	11	20.00%
GO:0030139	endocytic vesicle	GO:0005575	intracellular	7	12.73%
GO:0016787	hydrolase activity	GO:0005622	cell	5	9.09%
GO:0032555	purine ribonucleotide binding	GO:0005623	nucleotide binding	5	9.09%
GO:0097708	intracellular vesicle	GO:0000166	cytoplasm	4	7.27%
GO:0017076	purine nucleotide binding	GO:0005737	catalytic activity	3	5.45%
GO:0097367	carbohydrate derivative binding	GO:0003824	cytoskeleton	2	3.64%
GO:0035639	purine ribonucleoside triphosphate binding	GO:0005856	cytoskeletal protein binding	1	1.82%
GO:0032553	ribonucleotide binding	GO:0008092	actin binding	1	1.82%
GO:0031410	cytoplasmic vesicle	GO:0003779	protein binding	1	1.82%
GO:1901265	nucleoside phosphate binding	GO:0005515	hydrolase activity	1	1.82%
GO:0000166	nucleotide binding		Total	55	100.00%
GO:0003824	catalytic activity				
GO:0036094	small molecule binding				
GO:0043168	anion binding				
GO:0044430	cytoskeletal part				
GO:0031982	vesicle				
GO:0043227	membrane-bounded organelle				
GO:0003779	actin binding				

3 Responses of the kelp *Saccharina latissima* (Phaeophyceae) to the warming Arctic: from physiology to transcriptomics

Responses of the kelp *Saccharina latissima* (Phaeophyceae) to the warming Arctic: from physiology to transcriptomics

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The Arctic region is currently facing substantial environmental changes due to global warming. Melting glaciers cause reduced salinity environments in coastal Arctic habitats, which may be stressful for kelp beds. To investigate the responses of the kelp *Saccharina latissima* to the warming Arctic, we studied the transcriptomic changes of *S. latissima* from Kongsfjorden (Svalbard, Norway) over a 24-hour exposure to two salinities (Absolute Salinity [S_A] 20 and 30) after a 7-day pre-acclimation at three temperatures (0, 8 and 15°C). In addition, corresponding physiological data were assessed during an 11-days salinity/temperature experiment. Growth and maximal quantum yield for photosystem II fluorescence were positively affected by increased temperature during acclimation, whereas hyposalinity caused negative effects at the last day of treatment. In contrast, hyposalinity induced marked changes on the transcriptomic level. Compared to the control (8°C – S_A 30), the 8°C – S_A 20 exhibited the highest number of differentially expressed genes (DEGs), followed by the 0°C – S_A 20. Comparisons indicate that *S. latissima* tends to convert its energy from primary metabolism (e.g. photosynthesis) to antioxidant activity under hyposaline stress. The increase in physiological performance at 15°C shows that *S. latissima* in the Arctic region can adjust and might even benefit from increased temperatures. However, in Arctic fjord environments its performance might become impaired by decreased salinity as a result of ice melting.

Introduction

The increase in atmospheric temperature will be most pronounced in the Arctic, according to the predictions of the Intergovernmental Panel on Climate Change (IPCC 2014). The average water temperature in the upper 50 m of Kongsfjorden (Svalbard, Norway) exceeded 6°C in summer 2012 (Dalpadado et al. 2016). Furthermore, the summer sea surface temperature in the Arctic region is predicted to increase by 3−4°C in 2080−2099 (Müller

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Abbreviations − A, antheraxanthin; Acc, accessory pigment pool; AOX, alternative oxidase CI confidence interval; DEG, differentially expressed gene; DPS, de-epoxidation state DW dry weight FC fold change; Fuc, fucoxanthin; F_v/F_m, maximal quantum yield of photosystem II GO Gene Ontology; GST, glutathione *S*-transferase; HSP, heat shock protein; PES, Provasoli enriched seawater; PGK, phosphoglycerate kinase; PS II, photosystem II; RGR, relative growth rate (day^{-1}); RM, repeated measures; ROS, reactive oxygen species; S_A, Absolute Salinity (g kg^{-1}); TPM, transcripts per million; V, violaxanthin; VAZ, xanthophyll cycle pigment pool; vBPO, vanadium-dependent bromoperoxidase; Z, zeaxanthin.

et al. 2009). This temperature increase in the Arctic will result in increased freshwater discharge because of ice melting, which may have considerable effects on the salinity regime in the inshore waters of glacial fjords (Peterson et al. 2002, Mernild et al. 2015, Murray et al. 2015). In Kongsfjorden, salinity can decrease to Absolute Salinity (S_A) 23 in the surface water because of the large discharge of melting water (Hanelt et al. 2001), and hyposaline conditions can extend down to about 20 m depth due to wind- and wave-induced vertical mixing (Karsten 2007, Fischer et al. 2017). Such extensive environmental changes in Arctic fjords may result in transformations of seaweed communities. According to Bartsch et al. (2016), the overall seaweed biomass in 2012/2013 was 1.6-fold higher than in 1996/1998 in Kongsfjorden with a particularly high biomass increase at shallow depths (2.5 m). Model prediction suggest an extend of polar to cold-temperate seaweeds into the High Arctic within the end of the 21st century (Müller et al. 2009, Krause-Jensen and Duarte 2014). Despite the general observation of increased seaweed biomass in the Arctic, species-specific responses to environmental changes are likely to vary given their different optimal temperature and salinity ranges (Wiencke et al. 2006, Eggert 2012, Karsten 2012).

Saccharina latissima (Linnaeus) C.E. Lane, C. Mayes, Druehl et G.W. Saunders is a large brown alga inhabiting the Northern Hemisphere and of high economic and ecological value (McHugh 2003, Christie et al. 2009). Owing to the strong phenotypic plasticity, *S. latissima* survives along wide latitudinal gradients (40–80°N) and occurs from the upper sublittoral to a lower depth limit of about 30 m (Lüning et al. 1990). In Europe, its southern limit of distribution is at the Northern coast of Portugal (Lüning et al. 1990, Müller et al. 2009). *Saccharina latissima* is growing optimally between 10 and 15°C (Fortes and Lüning 1980). Concerning salinity, *S. latissima* displays an optimum growth between S_A 23 and 31, with a strong reduction of growth at S_A 16 and high mortality below S_A 8 (Gerard et al. 1987, Karsten 2007, Spurkland and Iken 2011).

Kongsfjorden (Svalbard, Norway) is an intensively studied monitoring site, allowing to observe the effects of global warming in the Arctic (Hop et al. 2002, Svendsen et al. 2002, Wiencke 2004). The interactive effects of increased temperature combined with irradiation (Müller et al. 2008, 2012, Olischläger and Wiencke 2013, Parages et al. 2013) and ocean acidification (Olischläger et al. 2014, Gordillo et al. 2016, Iñiguez et al. 2016, Olischläger et al. 2017) on Arctic kelps from Kongsfjorden have been well studied in the last decades. However, the effects of salinity and its interactive effects with other factors (i.e. temperature) on polar seaweeds are poorly known (Karsten 2007, Fredersdorf et al. 2009). Previous studies in kelp focussed on growth, photosynthetic performance and other biochemical parameters as response variables to environmental stress (Aguilera et al. 2002, Bischof et al. 2002, Michler et al. 2002). Only few studies investigated the molecular processes underlying the physiological performance. In recent years, with the improvement of molecular approaches, transcriptomic analysis has become a powerful tool to identify the regulation of gene expression towards stress acclimation in algae (*Ectocarpus siliculosus:* Dittami et al. 2009, *Saccharina japonica:* Deng et al. 2012, Liu et al. 2014b, *Sargassum thunbergii:* Liu et al. 2014a). Heinrich et al. (2012b, 2015 and 2016) published a series of studies on transcriptomic analysis of acclimation to temperature and light stress in *S. latissima* using the microarray technique.

In this study, we examined the physiological and transcriptomic responses of *S. latissima* under different temperature and salinity regimes to evaluate its response to a warming Arctic. Our specific objective was to assess stressor specific (salinity/temperature) responses of growth and photophysiological properties and their regulation at the transcriptomic level. We hypothesized that *S. latissima* growth would rather benefit from increased temperature, but may be impaired by hyposaline conditions.

Materials and methods

Algal material

Young sporophytes of *S. latissima* were raised from stock gametophyte cultures at the Alfred-Wegener-Institute Helmholtz Centre for Polar and Marine Research (AWI; culture numbers: 3123, 3124), which were established from spores of fertile sporophytes collected in Kongsfjorden (78°58′N; 11°30′E; Svalbard, Norway). Male and female gametophytes were mixed and transferred to petri dishes [filled with sterile Provasoli Enriched Seawater (PES); Starr and Zeikus 1993]. After two weeks, juvenile sporophytes were transferred to aerated 5 l glass bottles and grown in PES for nearly three months. When sporophytes had a sufficient length (12.68 ± 2.54 cm, range: 9.5 to 17.6 cm) they were selected for the following experiment. Fertilization and pre-cultivation were performed at 8 ± 1°C and an irradiance of 20 μmol photons m^2 s^{-1} (Mitras Lightbar Daylight 150, GHL) in a 18:6 h light:dark period. The medium was changed twice per week.

Temperature and salinity experiment

Aerated 5 l culture bottles each containing 12 sporophytes (pseudo-replicates) were filled with sterile PES (S_A

30). For pre-acclimation to temperature (day 0–7) 10 bottles per temperature were subjected to three walk-in temperature-controlled rooms (0, 8 and $15 \pm 1°C$). After 7 days of pre-acclimation, the bottles in each temperature were randomly separated and exposed to two salinity conditions, (S_A 20 and S_A 30; n = 5 true replicates for each) for the experimental phase (day 7–18). Each combination of temperature and salinity had five bottles as independent replicates. Salinity, expressed as S_A, g kg^{-1}, was measured with a refractometer (Atago S-10E). The S_A 20 and S_A 30 seawater was achieved by adding deionized water into natural seawater. Afterwards the diluted seawater was sterilized and Provasoli solution was added. The treatment at 8°C and S_A 30 (hereafter referred to 8°C – S_A 30) was defined as the control condition. Irradiance was set to 20 µmol photons m^{-2} s^{-1} in an 18:6 h light:dark (LD) period and PES was changed twice per week. One sporophyte per replicate was spot-labeled with a Pasteur pipette for fresh weight measurements, two sporophytes per bottle were randomly harvested on day 7, 8 and 18 for RNA and pigment analyses, five sporophytes served for the determination of maximal quantum yield of photosystem II (PS II; F_v/F_m) measurements and as a back-up. Fresh weight was determined on day 0, 4, 7, 11, 14 and 18. F_v/F_m was measured on day 0, 4, 7, 8, 11, 14 and 18. Although samples have been taken at several time points, pigments were only analyzed from day 8 and 18, and RNA only from day 8 due to logistical reasons. Samples for RNA extraction and pigment analysis were immediately frozen in liquid nitrogen after collection and stored at −80°C until further processing.

Algal growth

Given the initial high variability of fresh weight among individuals, one sporophyte per replicate was spot-labeled to allow for repeated fresh weight measurements and morphology observations at different timepoints. For fresh weight measurements we used three biological replicates per treatment (n = 3) due to practical limitations. Sporophytes were gently dried from surplus water using paper towels and weighed on an analytical digital balance (Sartorius LA310S). All fresh weight measurements were normalized to initial fresh weight that was set to 100%. Relative growth rates (RGRs, day^{-1}) were calculated between day 0 and 7 (pre-acclimation phase) and between day 7 and 18 (experimental phase) according to the formula of Lüning et al. (1990): RGR (day^{-1}) = $100 \times \ln(W_1/W_2)/(T_1 - T_2)$. Where W_1 is the fresh weight (g) in time 1 (T_1), W_2 is the fresh weight (g) in time 2 (T_2), T_1 and T_2 are the time in days.

Chlorophyll fluorescence measurements

One sporophyte per replicate (n = 5) was randomly chosen from each bottle per treatment for the determination of F_v/F_m (Maxi-version of the Imaging-PAM; Pulse Amplitude Fluorometer, Heinz Walz GmbH) which was measured after 10 min of dark adaptation. The sporophytes were put back to each bottle after measurements. For further details see Nielsen and Nielsen (2008).

Pigment analyses

One sporophyte was randomly chosen from each replicate (n = 5) in each treatment on day 8 and day 18. Each frozen sample was lyophilised (24 hours, condensator at −55°C) and pulverized separately by a benchtop homogenizer for 20 s (FastPrep®-24, MP Biomedicals). Subsamples [17–36 mg dry weight (DW)] with 1 ml of ice-cold 90% acetone were stored in the dark at 4°C for 24 hours for extracting pigments. After cold-centrifugation (5°C, 5 min, 13 000g), the supernatants were filtered with a 45 µm nylon syringe filter (Nalgene®, Nalge Nunc International) and transferred into HPLC glass vials. Pigments were analyzed by reversed-phase HPLC as described by Koch et al. (2015). Co-chromatography of standards (DHI LabProducts) for chlorophyll a and c2, fucoxanthin, β-carotene, violaxanthin, antheraxanthin and zeaxanthin were used to identify and quantify peaks detected at 440 nm. All extraction procedures were carried out under low light conditions.

The accessory pigment pool (Acc) was determined as the sum of chlorophyll c2 and fucoxanthin (Fuc). The xanthophyll cycle pigment pool (VAZ) was calculated by the sum of violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) concentrations. The de-epoxidation state (DPS) of xanthophyll cycle pigments was expressed according to Colombo-Pallotta et al. (2006): DPS=(Z+0.5A)/(V+A+Z).

Statistical analyses of physiological data

The differences of initial fresh weights between treatments were analyzed by a one-way ANOVA. With two one-way repeated measures (RM)-ANOVA we tested the effects of temperature on fresh weights and F_v/F_m of S. latissima during the pre-acclimation phase. Two two-way RM-ANOVA tested interactions of temperature and salinity on fresh weights and F_v/F_m of S. latissima during the experimental phase. The effects of temperature on RGRs from day 0 to day 7 were analyzed by a one-way ANOVA. A two-way ANOVA was used to test the effect of RGRs from day 7 to day 18. Since several data points in pigment results had abnormal distributions or heterogeneous variances, instead of RM-ANOVA, a two-way ANOVA was used

to test the effects of temperature and salinity on pigment contents and DPS of *S. latissima* on day 8 and day 18 separately.

Significant differences were further analyzed by pairwise comparisons with the Bonferroni adjustment for multiple comparisons. The statistical significance was accepted at a *P*-value below 0.05. RM-ANOVA's assumption of sphericity was checked by the Mauchly's test of sphericity. If sphericity was violated (i.e., when $P \leq 0.05$), epsilon (ε) was calculated according to Greenhouse and Geisser (1959) and used to correct the results of the RM-ANOVA. Normality and homogeneity of variances was checked with the Shapiro–Wilk and Levene's test. Since the assumptions were not met in Acc and DPS on day 8, as well as in RGRs from day 7 to day 18 for the salinity treatment, non-parametric tests were used (Mann–Whitney *U* test for salinity treatment, Kruskal-Wallis test for temperature treatment). All data are given as mean $\pm$ SD unless otherwise stated. Statistical analyses were performed using SPSS software version 25 (IBM).

RNA extraction, cDNA library construction and RNA-Seq

One sporophyte per replicate (n = 3) and treatment was randomly chosen on day 8 for RNA analysis. Total RNA extraction followed the protocol of Heinrich et al. (2012a). Quantity and purity of the extracted RNA was assessed by a Nanodrop ND-1000 Spectrophotometer (ThermoFisher), integrity of total RNA was determined by automated capillary electrophoresis using an Agilent 2100 Bioanalyzer (Agilent Technologies). The cDNA libraries were constructed by poly(A) enrichment using the Illumina TruSeq RNA Library Prep Kit (Illumina). The libraries were sequenced on an Illumina Hiseq 2500 instrument and 75 bp paired reads were clipped using default values of the Illumina software. The raw data were deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-7348.

Transcriptomic data processing

Quality checks of raw data were performed with FastQC v. 0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Low quality trimming was performed with Trimmomatic v. 0.36 (Bolger et al. 2014) using the following parameters: leading 3, trailing 3, sliding window 4:15, minlen 30. Remaining sequences were pseudo-aligned with Salmon (Patro et al. 2017) against an existing de novo reference transcriptome based on cDNA libraries of *S. latissima* from Roscoff, France (raw

data: E-MTAB-7348; Monteiro et al., unpublished data). The assembly was generated with Trinity v. 2.4.0 using the default parameters (Grabherr et al. 2011), quality evaluation of the assembled transcriptome was performed by BUSCO v2.0 (Waterhouse et al. 2017) with the Eukaryota dataset *odb9* (OrthoDB v9.1). The assembled transcriptome was compared to the bacterial and oomycete sequences of Genbank, by sequence similarity search (blastn), to investigate potential contamination. Differential gene expression was performed with DESeq2 (Love et al. 2014) using $8°C - S_A 30$ as reference sample. Trinity genes with an adjusted *P*-value of ≤ 0.001 and a $\log_2$ fold change (FC) of at least two were accepted as significantly differential expression (hereafter referred as 'genes'). Tools were applied using the Trinity package v. 2.4.0 (Grabherr et al. 2011). To explore the constitutively expressed transcripts within the control, normalized read counts, given as transcripts per million (TPM), were analyzed as described by Iñiguez et al. (2017). The Trinotate functional annotation pipeline (https://trinotate.github.io/) was used to perform the functional annotation. Venn diagrams were produced using the online tool http://bioinformatics.psb.ugent.be/webtools/Venn/. Gene Ontology (GO) enrichments were analyzed using GOseq (Young et al. 2010). Enriched GO terms were summarized with CateGOrizer using the EGAD2GO classification file (Hu et al. 2008).

Results

Fresh weights and relative growth rates

Pre-acclimation phase: For obtaining better comparability between different treatments, all fresh weights measured were calculated as percentage of initial fresh weights on day 0 (Fig. 1). There was no significant difference of initial fresh weights between different treatments ($P = 0.109$, one-way ANOVA). During seven days of temperature pre-acclimation, time had a significant effect on the overall increase in fresh weight ($P < 0.001$, Table S1) but temperature had no significant effect ($P = 0.078$). Additionally, the RGRs between the three experimental temperatures were not significantly different after seven days' pre-acclimation ($P = 0.095$; Fig. S2), with an average value from 8.79 day^{-1} to 14.31 day^{-1}.

Experimental phase: During the 11 days of salinity×temperatures treatments, fresh weights were significantly affected by time, temperature and the interaction of time×temperature and time × salinity (Table S2). At 0°C, the fresh weight did not increase significantly with time regardless of the salinity treatment. In contrast, at 8 and 15°C fresh weights significantly increased between day 7 to day 18. Temperature affected the fresh weight of samples on day 11, 14 and 18. On day 11
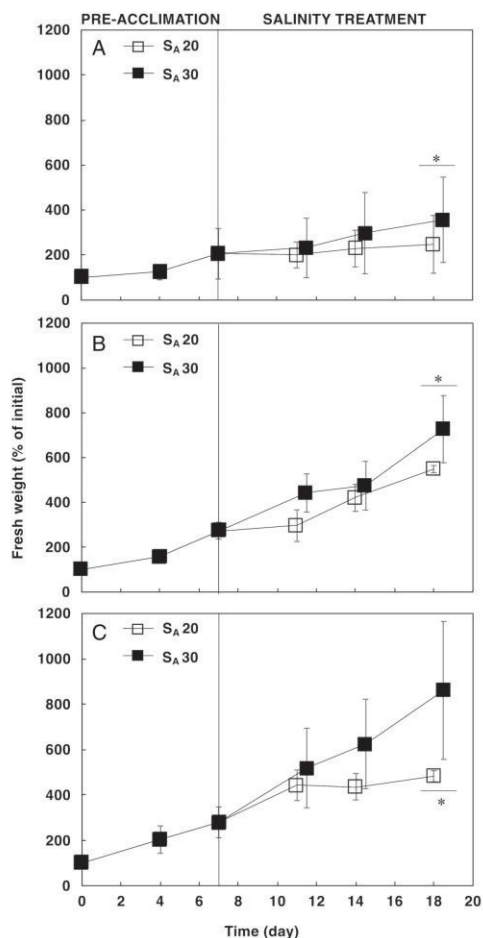
Fig. 1. Fresh weights of *Saccharina latissima* after 0, 4, 7, 11, 14 and 18 days of experiment at 0°C (A), 8°C (B) and 15°C (C) (100% of initial weights; mean ± standard deviation; n = 3). On the left, pre-acclimation phase – 7 days at 0, 8, 15°C; and on the right, salinity treatment – 11 days in S_A 20, 30 within 0, 8, 15°C. Significant differences between two salinities are given by asterisks (RM-ANOVA, $P \leq 0.05$).

and 14, sporophytes at 15°C were significantly heavier than at 0°C. On day 18, fresh weight at 8 and 15°C was significantly higher than at 0°C. Low salinity caused a significant reduction in growth of sporophytes at the end of the experiment irrespective of temperature (day 18). The overall mean fresh weight in S_A 30 was 221% [95% confidence interval (CI), 49 to 393] higher than in S_A 20 ($P = 0.016$). RGRs after

11 days of salinity treatment were significantly affected by temperature ($P = 0.022$, Kruskal-Wallis test) and salinity ($P = 0.011$, Mann-Whitney U-test; Fig. S2). RGR was significantly higher at 8°C (7.61 ± 1.5 day^{-1}) than at 0°C (3.39 ± 2.61 day^{-1}), and it increased by nearly 47% in S_A 30 compared to S_A 20 (from 4.32 ± 2.56 day^{-1} to 8.14 ± 3.79 day^{-1}).

Photophysiology and pigments

The maximal quantum yield of PS II (F_v/F_m) of *S. latissima* during seven days of pre-acclimation to temperature and subsequent 11 experimental days is shown in Fig. 2. From day 0 to day 7 (temperature pre-acclimation), F_v/F_m values showed a significant temperature × time interaction (Table S3). On day 4, F_v/F_m at 8°C was significantly lower than at 0°C, then F_v/F_m at 8°C increased and had a higher value than at 0°C on day 7. From day 7 to day 18 (salinity treatment within three temperatures), there was an interaction of time, temperature and salinity on F_v/F_m (Table S4). On day 8, after 24 h exposure to the combination of temperature and salinity, F_v/F_m was only affected by temperature ($P < 0.001$), not by salinity ($P = 0.496$) nor the interaction of temperature and salinity ($P = 0.673$). F_v/F_m at 8 and 15°C was significantly higher than at 0°C. On day 18, temperature and salinity showed a significant interaction ($P = 0.001$), at 15°C F_v/F_m was significantly lower under S_A 20 than under S_A 30, but not at 0 and 8°C.

The pigment contents and DPS after 24 h exposure (day 8) to salinity stress at three temperatures are exhibited in Fig. 3. The chlorophyll *a* and DPS content were neither affected by temperature nor by salinity (Table S5). In contrast, the higher temperature caused a significant increase of the accessory pigment pool (Acc; $P = 0.001$, Kruskal–Wallis test) while salinity had no effect on Acc ($P = 0.290$, Mann-Whitney U-test). Furthermore, the xanthophyll-cycle pool (VAZ) was significantly affected by temperature and salinity, but there was no interaction between factors. The overall content of VAZ in S_A 20 was 0.033 (95% CI, 0.003 to 0.062) µg mg^{-1} DW higher than the content in S_A 30 ($P = 0.03$). Overall VAZ at 15°C was significantly higher than at 0°C ($P = 0.008$) and 8°C ($P = 0.006$). On day 18, chlorophyll *a* content remained similar in different treatments (Table S6, Fig. S3). DPS at 0°C (0.084 ± 0.03) was significantly higher than at 8°C (0.042 ± 0.012) and 15°C (0.027 ± 0.008), while DPS in S_A 20 had a higher value than in S_A 30.

Gene expression

Sequencing yielded 27.4 to 35.8 million reads per sample, with an average of 32.8 million reads.
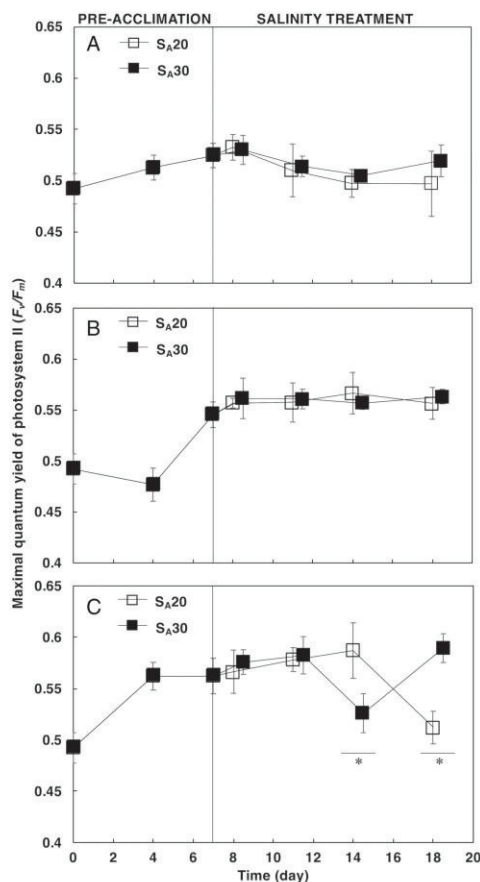
Fig. 2. Maximal quantum yield of photosystem II (F_v/F_m) of *Saccharina latissima* after 0, 4, 7, 8, 11, 14 and 18 days of experiment at 0°C (A), 8°C (B) and 15°C (C; mean ± SD; n = 5). On the left, pre-acclimation phase – 7 days at 0, 8, 15°C; and on the right, salinity treatment – 11 days in S_A 20, 30 within 0, 8, 15°C. Asterisks stand for the significant differences between two salinities (RM-ANOVA, $P \leq 0.05$).

Approximately 85.83 ± 1.16% of the reads could be aligned to the reference transcriptome. Among 135 959 genes, 4026 (2.96%) showed significantly different regulation under at least one treatment compared to the control group (8°C – S_A 30). The 8°C – S_A 20 induced the strongest effect in gene expression with 1374 differentially expressed genes (DEGs), followed by the 0°C – S_A 20 (1193 DEGs; Fig. 4). The 15°C – S_A 20, however, featured a lower number of DEGs (384) than the 15°C – S_A 30 (801). The lowest number of DEGs

was observed in the 0°C – S_A 30 (274). In all treatments, more downregulated genes than upregulated genes were observed. Overall, expression patterns at 0 and 15°C in S_A 20 and S_A 30 suggest interacting effects of temperature and salinity on the number of DEGs (Fig. 4).

The DEGs of different treatments vs the control group were compared using Venn diagrams to identify overlapping DEGs (Fig. 5). There was a small proportion of DEGs commonly regulated among the treatments. In S_A 20, three upregulated and 13 downregulated DEGs were shared within the three different temperatures. 8°C – S_A 20 and 0°C – S_A 20 shared more DEGs than 8°C – S_A 20 and 15°C – S_A 20, and 15°C – S_A 20 and 0°C – S_A 20. 0°C – S_A 30 and 15°C – S_A 30 shared six upregulated and 32 downregulated genes. 15°C promoted more downregulated genes (559) than upregulated ones (204). In contrast, 0°C only had 126 upregulated genes and a similar number of downregulated genes (110).

GO enrichment analysis

GO enrichment analysis was conducted for each comparison to the control treatment. Table 1 shows the number of over-represented GO terms within the three GO root categories. The highest number of enriched GO terms was observed in 8°C – S_A 20, followed by 0°C – S_A 20. 0°C – S_A 30 showed relatively more enriched GO terms (374) compared to its small number of DEGs (274). Downregulated genes featured more enriched GO terms than upregulated genes in all different treatments, especially 8°C – S_A 20 with 49 GO terms in upregulated genes vs 404 GO terms in downregulated genes.

In Fig. 6 the functional comparison derived from enriched GO terms of upregulated genes in the five treatments is shown. Both 8°C – S_A 20 and 0°C – S_A 20 induced a strong regulation of transport-related genes, accounting for the highest percentage. In contrast, transport genes were less represented in 15°C – S_A 20. However, high temperature (15°C) triggered intense regulation of signaling genes in both salinity treatments. Moreover, 15°C also caused an enhanced regulation of genes belonging to carbohydrate metabolism, stress response and protein modification, which were rarely regulated at 0 and 8°C. Furthermore, low temperature (0°C) induced more changes related to lipid metabolism and cell structure than the 15°C treatments. For downregulated genes, the number of different categories derived from enriched GO terms was different from the upregulated genes (Fig. S4). The repression of genes related to signaling accounted for the highest proportion at both 0 and 15°C. In contrast, most downregulated genes
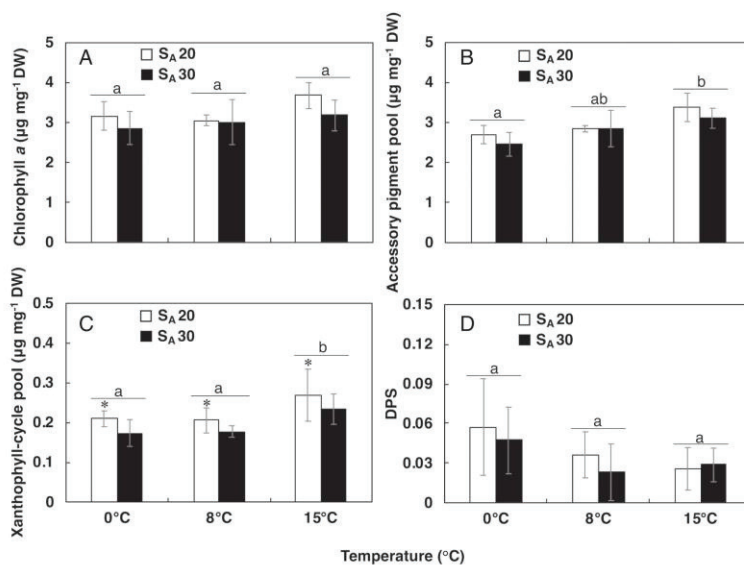
Fig. 3. Chlorophyll *a* content (A), accessory pigment pool content (Acc; B), xanthophyll-cycle pool content (VAZ; C) (μg mg^{-1} DW; mean $\pm$ standard deviation; n = 5) and de-epoxidation states of xanthophyll cycle (DPS; D) (mean $\pm$ standard deviation; n = 5) of *Saccharina latissima* after exposure to two salinity conditions (S_A 20, 30) within three temperatures (0, 8, 15°C) on day 8. Significant differences between temperature irrespective of salinity are given by small letters and differences between salinities within temperature treatments are shown by asterisks (two-way ANOVA, $P \leq 0.05$).
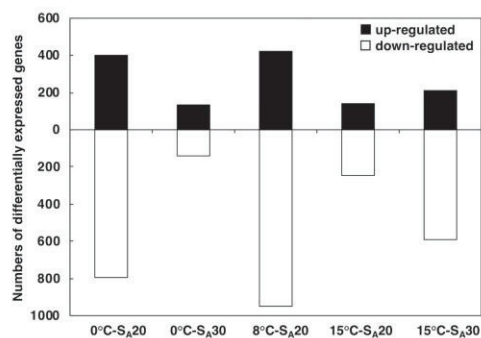
Fig. 4. Number of significantly different up (black bars) and downregulated (white bars) DEGs in *Saccharina latissima* after 24 h exposure to two salinity conditions (S_A 20, 30) at three temperatures (0, 8, 15°C) compared to the control (8°C – S_A 30; $P \leq 0.001$, Log$_2$ FC $\geq$2).

belonged to metabolism in 8°C – S_A 20, with only three terms related to signaling.

Manual inspection of potential stress responsive genes

To investigate the transcriptomic responses of *S. latissima* to temperature and salinity variation in more detail, we manually analyzed DEGs involved in photosynthesis-related metabolism, general stress

responses, cellular transport and potential osmolytes (Tables 3,4 and 5). Table 2 shows the numbers of DEGs belonging to each of these four categories. A complete list of DEGs with annotations is available in the Supporting Information (Table S8).

Photosynthesis and pigment metabolism

The treatment 8°C – S_A 20 caused a repression of most DEGs, with a Log$_2$ FC of around 2, coding for enzymes involved in chlorophyll biosynthesis (e.g. glutamate-1-semialdehyde 2,1-aminomutase, porphobilinogen deaminase and uroporphyrinogen decarboxylase), chlorophyll catabolism (i.e. phaeophytinase and phaeophorbide a oxygenase), carotenoid biosynthesis (e.g. zeaxanthin epoxidase and violaxanthin de-epoxidase), Calvin cycle (e.g. chloroplastic fructose-1,6-bisphosphatase), photosynthetic electron transport chain (e.g. ferredoxin-1 and photosynthetic NDH subunit of subcomplex B3) and the light harvesting complex (e.g. fucoxanthin-chlorophyll *a-c* binding protein D).

The treatments 0°C – S_A 20 and 0°C – S_A 30 repressed the DEG encoding chloroplastic phosphoglycerate kinase (PGK) involved in the Calvin cycle, while DEGs related to chlorophyll and carotenoid biosynthesis were induced with a log$_2$ FC of 2 to 3 (i.e. chlorophyllide a oxygenase and ubiquinol oxidase 4; Table 3).
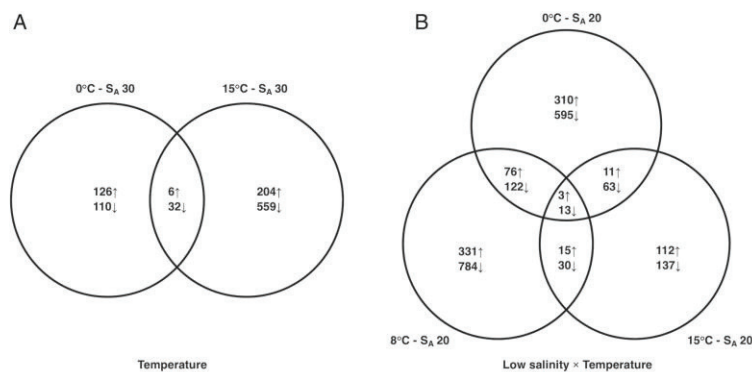
Fig. 5. Venn diagrams of significantly upregulated (↑) and downregulated (↓) DEGs in *Saccharina latissima* after 24 h exposure to 0°C – S_A 30 and 15°C – S_A 30 (A), as well as 0°C – S_A 20, 8°C – S_A 20 and 15°C – S_A 20 (B) compared to the control (8°C – S_A 30; $P \leq 0.001$, Log_2 FC ≥ 2).

Table 1. Numbers of over-represented GO terms within the three GO root categories: cellular components, molecular function and biological process among different treatments. Each treatment was compared to the control (8°C – S_A 30).

Treatments	Regulation	GO root category			
		Cellular component	Molecular function	Biological process	Total
0°C – S_A 20	Up	25	35	86	146
	Down	15	60	183	258
0°C – S_A 30	Up	29	29	72	130
	Down	12	36	196	244
8°C – S_A 20	Up	8	17	24	49
	Down	61	104	239	404
15°C – S_A 20	Up	2	17	28	47
	Down	12	25	53	90
15°C – S_A 30	Up	1	13	30	44
	Down	12	65	109	186

General stress responses

Several DEGs encoding glutathione *S*-transferase were upregulated at 0°C under both S_A 20 and S_A 30 with a Log_2 FC of 2–3 (Table 4). Furthermore, 0°C – S_A 20 induced one DEG encoding peptide methionine sulfoxide reductase MsrA and one DEG encoding mitochondrial alternative oxidase (AOX). In 8°C – S_A 20, there were five upregulated genes and eight downregulated genes related to the response to general stress (Table 2). Five DEGs encoding vanadium-dependent bromoperoxidase (vBPO) were approximately 2-Log_2 fold induced in 8°C – S_A 20. In contrast, 15°C – S_A 20 and 15°C – S_A 30 only showed 2- to 4-Log_2 fold downregulated genes related to stress responses, such as vBPO and glutathione *S*-transferase (GST). Besides, several DEGs were only downregulated in 8°C – S_A 20, e.g. superoxide dismutase (Fe), L-ascorbate peroxidase 6, chloroplastic peroxiredoxin Q and peroxiredoxin-2E-1.

Two DEGs were related to heat shock proteins (HSPs). One DEG encoding 78 kDa glucose-regulated protein was downregulated in 15°C – S_A 30 and another DEG encoding chaperone protein ClpB 2 was repressed in 8°C – S_A 20.

Cellular transport and potential osmolytes

We detected a significant regulation of genes encoding proteins involved in transport processes. 15°C mainly caused the downregulation of genes, except the induction of cell wall protein PRY3 in 15°C – S_A 20 (Table 5). 8°C – S_A 20 triggered the highest number of regulated transcripts, with five upregulated and 22 downregulated genes (Table 2). Three DEGs from the ABC transporter family were 2-Log_2 fold induced in 0°C – S_A 20, while another DEG for the ABC transporter was repressed in the same conditions (Table 5). 8°C – S_A 20 also lead to changes in regulation of ABC transporters, with two DEGs 2- to 3-Log_2 fold upregulated and one DEG 2-Log_2 fold downregulated. In contrast, 15°C featured only downregulated DEGs from the ABC transporters family in both S_A 20 and S_A 30. Furthermore, 15°C showed a downregulated DEG encoding an aquaporin. One DEG encoding a potassium channel was 2-Log_2 fold downregulated in 0°C – S_A 20. Regarding potential osmolytes, we failed to find any gene regulation related to mannitol metabolism. Although we detected the occurrence of a gene encoding mannitol 2-dehydrogenase, it was not differently expressed between treatments and the control. Its expression in the control was 59.69 TPM (Table S7). In contrast, genes coding for choline dehydrogenase, which is involved in the biosynthesis of the osmoprotectant glycine betaine, were downregulated in 0°C – S_A 20 and 8°C – S_A 20, but not in 15°C – S_A 20.
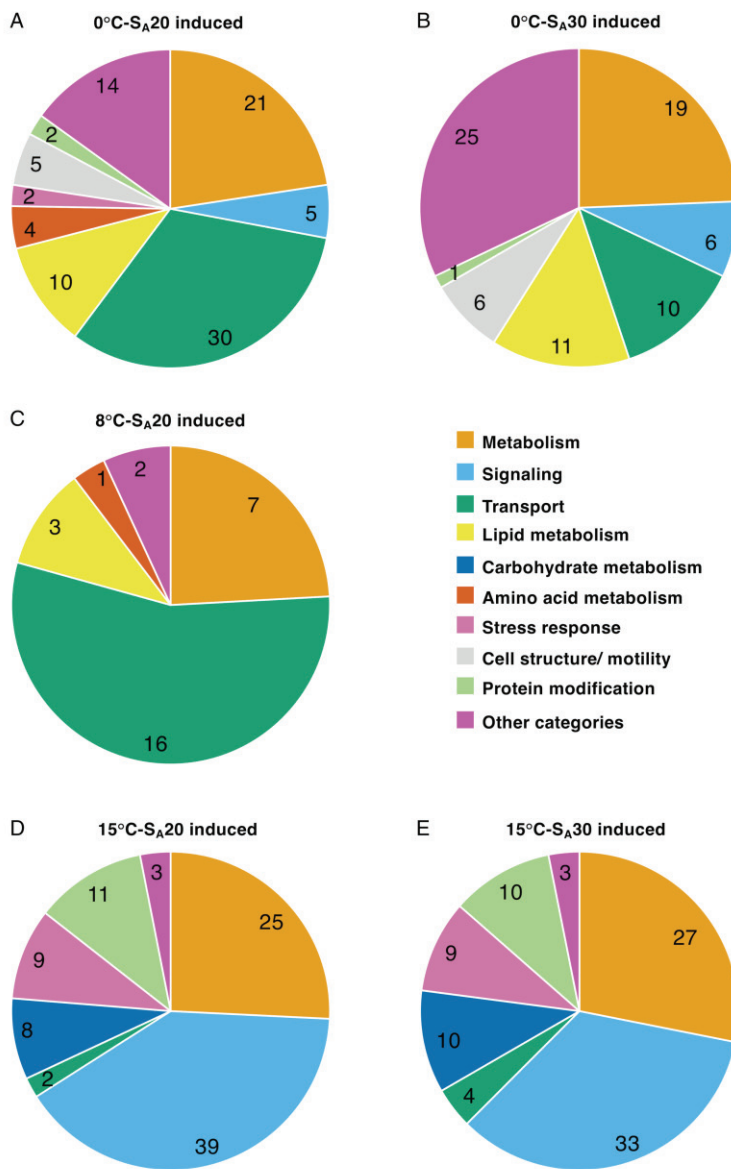
Fig. 6. Functional categories derived from enriched GO terms of upregulated genes of treatment $0°C - S_A$ 20 (A), $0°C - S_A$ 30 (B), $8°C - S_A$ 20 (C), $15°C - S_A$ 20 (D) and $15°C - S_A$ 30 (E) compared to the control ($8°C - S_A$ 30): classification after EGAD2GO using cateGOrizer.

Discussion

Our results showed that 2.96% of the expressed gene fragments had a significantly altered expression under different salinity and temperature treatments compared to the control. One of the reasons leading to the low proportion of DEGs is likely the amplitude of stress exposure, which in our study was not at the extremes of physiological tolerance of *S. latissima*. Besides, exposure time also modulates transcriptomic regulation, as experiments

Table 2. Numbers of differentially expressed genes (DEGs) belonging to the functional categories of photosynthesis related metabolism, general stress responses, cellular transport and potential osmolytes, assigned by manual classification. Each treatment was compared to the control group (8°C − S_A 30; $P \leq 0.001$, Log_2 FC $\geq$2).

Putative gene products	Regulation	0°C – S_A 20	0°C – S_A 30	8°C – S_A 20	15°C – S_A 20	15°C – S_A 30
Photosynthesis related metabolism	Up	3	3	0	0	1
	Down	4	2	33	0	0
General stress responses	Up	5	3	5	0	0
	Down	2	0	8	9	13
Cellular transport	Up	6	2	5	1	0
	Down	9	1	22	9	11
Potential osmolytes	Up	0	0	0	0	0
	Down	2	1	3	0	0

in land plants suggested that the most marked changes in transcription occur after 6 hours of stress exposure (Seki et al. 2002). Before low salinity exposure, we conducted a 7-day temperature pre-acclimation. This allowed for the adjustment of sporophytes to different temperatures and may explain the low number of transcriptomic changes induced by temperature. When comparing the number of DEGs between different treatments, the 8°C – S_A 20 featured the highest number of DEGs, followed by the 0°C – S_A 20, although salinity did not cause differences in most of the physiological parameters after 24 h of salinity stress. The apparent reallocation of resources on the transcriptomic level may then eventually impair individual fitness in the long run, which might be reflected by the lower fresh weights in S_A 20 relative to S_A 30 on day 18. A previous study showed that low salinity was positively correlated with low dry matter content in *S. latissima*, reflecting the energy transferred from biomass production to osmoregulation at low salinity (Nielsen et al. 2016). Moreover, the number of DEGs in 15°C – S_A 20 was lower than in 0°C – S_A 20 and 8°C – S_A 20, suggesting that increased temperature can alleviate short-term responses of *S. latissima* to low salinity stress. Because the transcriptome changes continuously over time (Kahl 2015), the long-term acclimation responses of sporophytes on the transcriptomic level on day 18 (after 11 days of salinity treatment) is expected to be different from short-term stress responses on day 8, after 24 hours of exposure. In the following transcriptomic analysis, we focus on the short-term responses of *S. latissima* to decreased salinity, because in situ salinity drops can occur rapidly in Arctic fjords due to freshwater run-off. In addition, physiological acclimation responses are considered over the whole 18 days course of the experiment.

Photosynthetic responses

Our physiological data show that increased temperature promotes the biosynthesis of accessory pigments and an increase in F_v/F_m. Differences in pigmentation of *S. latissima* at different temperature regimes were reflected by the mere color of sporophytes. After 18 days of treatment, the color of *S. latissima* grown at 15°C was dark brown, while slightly lightened at 8°C and turned to light brown at 0°C (Fig. S1). The accessory pigments of the light harvesting complex are mainly responsible for the color of algae, particularly fucoxanthin accounting for the typically brown color of the Phaeophyceae (Hanelt et al. 2003). Hence, the higher Acc in 15°C – S_A 20 and 8°C – S_A 20 compared to 0°C – S_A 20 (Fig. S3) could partially explain the differences in color. This was also observed by Davison et al. (1991). *S. latissima* grown at 15° became darker than 5°C sporophytes due to the increase of all three major photosynthetic pigments (chlorophyll *a*, chlorophyll *c* and fucoxanthin). This direct relationship between pigment content and temperature is well known for many other algae and macrophytes (Marsh et al. 1986, Geider 1987) and might be related to increased photosynthetic rates, likely to match increased Calvin cycle activity at higher temperature. This increase of pigment contents at higher temperatures, leading to higher photosynthesis, might ultimately promote growth, which was reflected by higher growth rates at elevated temperatures. Salinity also had a severe effect on pigmentation. Severe bleaching occurred in the distal thallus parts of *S. latissima* in 0°C – S_A 20. This was less pronounced in 8°C – S_A 20 and only occurred in the blade tips of *S. latissima* in 15°C – S_A 20. Possibly, protective processes might be less efficient at low temperature. Such pigment bleaching also occurred in the field in Kongsfjorden, where *S. latissima* appeared greenish when exposed to freshwater run-off (Karsten 2007).

The DPS reflects the photoprotective state of the xanthophyll cycle. The more antheraxanthin and zeaxanthin are involved, the more energy will be dissipated as heat, conferring photoprotection to the photosynthetic apparatus (Pfündel and Bilger 1994). In our study, the differences in DPS between temperatures and salinities

Table 3. Selected significant DEGs with functions in regulating photosynthetic components, Calvin cycle and pigments metabolism as compared to the control group (8°C – S_A 30; $P \leq 0.001$, Log_2 FC $\geq$2).

Gene ID	Putative gene product	Annotation e-value	Log₂ fold change				
			$0°C - S_A\ 20$	$0°C - S_A\ 30$	$8°C - S_A\ 20$	$15°C - S_A\ 20$	$15°C - S_A\ 30$
Light-harvesting chlorophyll protein complex/antenna proteins							
TRINITY_DN30327_c7_g2	Chlorophyll *a-b* binding protein L1818, chloroplastic	3.30E-10	-	-	-	-	2.16
TRINITY_DN29722_c7_g2	Fucoxanthin–chlorophyll *a-c* binding protein D, chloroplastic	1.80E-40	-	-	-2.08	-	-
Photosynthetic electron transport chain							
TRINITY_DN24625_c0_g1	Ferredoxin-1	3.10E-14	-	-	-2.34	-	-
TRINITY_DN30332_c3_g1	Photosynthetic NDH subunit of subcomplex B 3, chloroplastic	1.80E-16	-	-	-2.18	-	-
TRINITY_DN27962_c7_g6	PsbB mRNA maturation factor Mbb1, chloroplastic	1.90E-37	-	-	-2.05	-	-
Calvin cycle/carbon fixation							
TRINITY_DN28988_c5_g2	Phosphoglycerate kinase, chloroplastic	5.30E-65	-2.60	-2.13	-	-	-
TRINITY_DN27519_c12_g1	Ribulose-phosphate 3-epimerase	1.9E-39	-	-	-2.32	-	-
TRINITY_DN8663_c0_g1	Ribulose-1,5 bisphosphate carboxylase/oxygenase large subunit *N*-methyltransferase, chloroplastic	1.7E-42	-	-	-2.29	-	-
TRINITY_DN25611_c0_g1	Fructose-1,6-bisphosphatase, chloroplastic	8.90E-58	-	-	-2.13	-	-
Assembly of the photosystem II							
TRINITY_DN26599_c6_g2	Carboxyl-terminal-processing peptidase 1, chloroplastic	9E-52	-	-	-2.22	-	-
Chlorophyll biosynthesis							
TRINITY_DN27182_c3_g1	Chlorophyllide a oxygenase, chloroplastic	1.80E-11	2.53	2.28	-	-	-
TRINITY_DN27996_c6_g1	Glutamate-1-semialdehyde 2,1-aminomutase, chloroplastic	2.80E-148	-	-	-3.13	-	-
TRINITY_DN27889_c3_g2	Uroporphyrinogen decarboxylase	1.60E-96	-	-	-2.32	-	-
TRINITY_DN27073_c11_g1	Protochlorophyllide reductase, chloroplastic	8.50E-46	-	-	-2.68	-	-
TRINITY_DN27926_c9_g4	Divinyl chlorophyllide a 8-vinyl-reductase, chloroplastic	1.60E-74	-	-	-2.64	-	-
TRINITY_DN28273_c1_g1	Porphobilinogen deaminase, chloroplastic	1.20E-102	-	-	-2.11	-	-
TRINITY_DN29151_c2_g1	Geranylgeranyl diphosphate reductase	2.50E-137	-	-	-2.68	-	-
TRINITY_DN27421_c2_g2	Magnesium protoporphyrin IX methyltransferase, chloroplastic	9.80E-69	-	-	-2.12	-	-
Carotenoid biosynthesis							
TRINITY_DN28566_c5_g2	Zeaxanthin epoxidase, chloroplastic	2.50E-15	-	-	-2.05	-	-
TRINITY_DN27115_c9_g2	Violaxanthin de-epoxidase, chloroplastic	4.10E-22	-	-	-2.02	-	-
TRINITY_DN27339_c6_g1	Phytoene synthase, chloroplastic	6.10E-81	-	-	-2.01	-	-
TRINITY_DN26187_c0_g2	Cytochrome P450 97B3, chloroplastic	5.40E-155	-	-	-2.44	-	-
TRINITY_DN28191_c5_g1	Beta-carotene isomerase D27, chloroplastic	2.20E-19	-	-	-2.36	-	-
TRINITY_DN26365_c9_g2	Homogentisate solanesyltransferase, chloroplastic	6.40E-71	-	-	-2.53	-	-
TRINITY_DN28232_c5_g2	Prolycopene isomerase, chloroplastic	4.60E-42	-	-	-2.73	-	-
TRINITY_DN26248_c0_g1	Ubiquinol oxidase 4, chloroplastic/chromoplastic	1.50E-48	2.83	2.74	-	-	-
TRINITY_DN26338_c9_g1	Ubiquinol oxidase 4, chloroplastic/chromoplastic	8.30E-24	4.36	3.78	-	-	-
Chlorophyll catabolism							
TRINITY_DN25286_c1_g1	Pheophytinase, chloroplastic	8.10E-27	-	-	-2.09	-	-
TRINITY_DN27051_c6_g1	Pheophorbide a oxygenase, chloroplastic	2.50E-19	-	-	-2.05	-	-

Table 4. Selected significant DEGs with functions in regulating general stress responses as compared to the control group (8°C – S$_A$ 30; $P \leq 0.001$, Log$_2$ FC $\geq$2).

Gene ID	Putative gene product	Annotation e-value	Log$_2$ Fold change				
			0°C – S$_A$ 20	0°C – S$_A$ 30	8°C- S$_A$ 20	15°C- S$_A$ 20	15°C- S$_A$ 30
TRINITY_DN30059_c1_g4	Vanadium-dependent bromoperoxidase	3.50E-11	–2.10	-	2.60	–2.51	–2.15
TRINITY_DN27187_c6_g2	Vanadium-dependent bromoperoxidase	4.70E-91	-	-	-	–2.68	–3.08
TRINITY_DN30022_c1_g9	Vanadium-dependent bromoperoxidase	1.10E-11	-	-	-	-	–2.11
TRINITY_DN27187_c7_g1	Vanadium-dependent bromoperoxidase	2.60E-25	-	-	-	–2.49	–2.48
TRINITY_DN30390_c2_g1	Vanadium-dependent bromoperoxidase	2.50E-90	-	-	2.30	-	–3.66
TRINITY_DN30053_c6_g5	Vanadium-dependent bromoperoxidase	2.50E-73	-	-	-	–2.65	–2.94
TRINITY_DN30345_c4_g3	Vanadium-dependent bromoperoxidase	1.80E-19	-	-	2.31	-	–3.63
TRINITY_DN26995_c10_g2	Vanadium-dependent bromoperoxidase	1.60E-11	-	-	2.84	-	–2.49
TRINITY_DN30510_c0_g1	Vanadium-dependent bromoperoxidase	6.00E-25	-	-	2.21	-	-
TRINITY_DN28519_c1_g3	Glutathione S-transferase	4.60E-16	3.92	2.67	-	–2.32	–3.03
TRINITY_DN29794_c0_g11	Glutathione S-transferase	7.90E-11	3.75	2.82	-	–3.67	–4.63
TRINITY_DN29486_c9_g1	Glutathione S-transferase	1.80E-21	2.17	2.19	-	–2.03	-
TRINITY_DN27888_c16_g1	Alternative oxidase, mitochondrial	4.00E-69	2.18	-	-	-	-
TRINITY_DN26569_c2_g2	Superoxide dismutase (Fe)	4.30E-37	-	-	–2.68	-	-
TRINITY_DN30350_c9_g3	ʟ-ascorbate peroxidase 6	1.80E-15	-	-	–2.40	-	-
TRINITY_DN26710_c13_g1	Peptide methionine sulfoxide reductase MsrA	2.70E-48	2.52	-	-	-	-
TRINITY_DN28339_c3_g1	Peroxiredoxin-2E-1, chloroplastic	4.60E-32	-	-	–2.29	-	-
TRINITY_DN25903_c0_g1	Peroxiredoxin Q	1.50E-35	-	-	–2.44	-	-
TRINITY_DN29226_c7_g3	Putative heme-binding peroxidase	2.40E-57	–2.05	-	–3.03	-	-
TRINITY_DN30350_c9_g3	Putative ʟ-ascorbate peroxidase 6	1.80E-15	-	-	–2.4	-	-
TRINITY_DN29558_c9_g4	78 kDa glucose-regulated protein	2.30E-221	-	-	-	-	–3.37
TRINITY_DN26603_c0_g1	Chaperone protein ClpB 2	6.30E-277	-	-	–2.16	-	-

Table 5. Selected significant DEGs with functions in regulating cellular transport and potential osmolytes as compared to the control group (8°C – S_A 30; $P \leq 0.001$, Log_2 FC ≥ 2).

Gene ID	Putative gene product	Annotation e-value	Log_2 Fold change				
			0°C – S_A 20	0°C - S_A 30	8°C - S_A 20	15°C - S_A 20	15°C - S_A 30
Cellular transport							
TRINITY_DN27652_c2_g7	Probable anion transporter 5	6.10E-12	–3.13	-	–2.34	-	-
TRINITY_DN25270_c0_g1	Solute carrier family 35 member F6	1.90E-26	-	-	–3.07	–2.56	-
TRINITY_DN27989_c9_g3	ABC transporter G family member 7	4.20E-65	-	-	–2.13	-	-
TRINITY_DN27882_c8_g4	ABC transporter G family member 6	1.30E-93	2.47	-	-	-	–3.84
TRINITY_DN26402_c10_g6	Serine protease/ABC transporter B family protein tagA	1.10E-20	-	-	2.19	-	–2.61
TRINITY_DN29513_c8_g2	Protein white	3.60E-61	2.37	-	-	–2.61	–2.61
TRINITY_DN30332_c3_g1	Protein white	1.80E-16	2.30	-	-	–2.43	–2.46
TRINITY_DN29832_c8_g2	ABC transporter G family member 15	1.30E-57	–2.09	-	-	-	-
TRINITY_DN29832_c8_g1	ABC transporter G family member 7	3.10E-73	-	-	3.75	-	-
TRINITY_DN29103_c8_g2	Protein zinc induced facilitator-like 1	1.90E-32	-	-	–3.55	–2.37	-
TRINITY_DN19005_c0_g1	Intraflagellar transport protein 52	1.60E-46	3.15	-	-	-	-
TRINITY_DN26341_c9_g2	Intraflagellar transport protein 56	7.00E-136	2.66	2.84	-	-	-
TRINITY_DN29908_c6_g5	Probable outer membrane protein pmp6	3.80E-11	-	-	-	–2.84	–2.53
TRINITY_DN27415_c3_g2	Two pore potassium channel c	8.30E-24	–2.22	-	-	-	-
TRINITY_DN26919_c0_g1	Cell wall protein PRY3	8.40E-11	-	-	-	2.41	-
TRINITY_DN27953_c13_g2	Probable aquaporin PIP1-2	1.20E-33	-	-	-	–2.26	–2.70
Potential osmolytes							
TRINITY_DN25054_c0_g1	Oxygen-dependent choline dehydrogenase	2.70E-69	–2.86	–2.05	–3.26	-	-
TRINITY_DN29689_c1_g2	Oxygen-dependent choline dehydrogenase	2.90E-13	–2.35	-	–2.61	-	-
TRINITY_DN29689_c1_g1	Oxygen-dependent choline dehydrogenase	9.80E-29	-	-	–2.38	-	-

increased from day 8 to day 18, indicating that regulatory responses developed with longer exposure time to low temperature and hyposaline stress. High DPS can protect PS II from photo-oxidative damage by increasing energy dissipation (Smith et al. 2010). As discussed by Jahnke and White (2003), the enlarged chloroplasts induced by low osmolarity can reduce the electron flow at the photosystem I donor side, leading to the over-reduction of the primary acceptor of PS II and increased formation of reactive oxygen species (ROS). On the other hand, enzymatic reactions are reduced by low temperatures, while primary reactions of photosynthesis are not (Hurd et al. 2014). Consequently, the absorbed energy that exceeds the capacity of electron transport can result in ROS formation (Bischof and Rautenberger 2012). The high DPS at 0°C might be responsible for the lower values of F_v/F_m. Demmig-Adams and Adams III (1996) proposed that the high levels of energy dissipation in the antennae reduce the overall efficiency of energy conversion in photosystem II, because less of the absorbed light is delivered to the photochemical reaction centres. The negative relationship between F_v/F_m and the de-epoxidation state of the xanthophyll cycle also became apparent under different abiotic stresses (e.g. high light and chilling temperatures; Demmig et al. 1988, Adams III et al. 1994).

Hyposalinity has negative impacts on photosynthesis of kelps, which is reflected by the decreased F_v/F_m (Karsten 2007, Spurkland and Iken 2011). However, F_v/F_m showed no significant difference between S_A 20 and S_A 30 in our study except for the fluctuations at 15°C on day 14 and 18. These results agree with findings from Spurkland and Iken (2011) for *S. latissima* grown in S_A 10 to S_A 31. After 18 days exposure, F_v/F_m remained similar in S_A 20 and S_A 31 and decreased significantly in S_A 10. Karsten (2007) also found that the effective quantum yield of *S. latissima* maintained values >80% of the control in S_A 25 to S_A 55, which decreased gradually from S_A 20 to S_A 10 on day 5. In contrast to the constant F_v/F_m in S_A 20, on the transcriptomic level a large set of genes related to photosynthesis in 8°C – S_A 20 were repressed, ranging from photosynthetic components to pigment synthesis and carbon fixation enzymes. Heinrich et al. (2015) reported similar results. They showed that *S. latissima* markedly changed its gene expression without variation in quantum yield under a set of temperature and UV radiation exposure conditions. We assume that this discrepancy between photosynthetic and transcriptomic response is due to a short-term transcriptomic regulation of *S. latissima* to acclimate to hyposalinity and might become reduced during prolongation of exposure (however data for day 11 of the salinity treatment have not been analyzed). In addition, the non-parallel performance between F_v/F_m and transcriptome responses

suggests that F_v/F_m might not always be an ideal parameter to detect for the onset of stress. As discussed by Kromkamp and Forster (2003), the maximal quantum yield of PS II does not necessarily represent the whole potential of photosynthesis in organisms, because the limitations might take place elsewhere in the photosynthetic process, not reflected by F_v/F_m, such as the Calvin cycle.

Repression of photosynthesis related transcripts by hyposaline conditions was also described in other macroalgae. Teo et al. (2009) observed differential regulation of genes encoding light-harvesting proteins (two downregulated and one upregulated) of *Gracilaria changii* under hypoosmotic stress for seven days. Dittami et al. (2009) found that the brown alga *E. siliculosus* under hyposaline stress had a clear upregulation of signaling, osmolytes, transporters and the downregulation of primary metabolism (e.g. photosynthesis and protein synthesis). A possible reason is that algae reallocate energy to keep their homeostasis under hyposaline stress, which was also stated by Davison and Pearson (1996). These transcriptomic changes in 8°C – S_A 20 were not mirrored in the physiological parameters explored in our study, however, they support the adverse effects reported from other physiological experiments, e.g. reducing chloroplast numbers (Iyer and Barnabas 1993) and decreasing photosynthetic activity (Gessner and Hammer 1960, Karsten 2007, Liu et al. 2012).

Compared to the large numbers of downregulated DEGs within the category photosynthesis in 8°C – S_A 20, the numbers of DEGs in 0°C – S_A 20 and 15°C – S_A 20 were relatively low. Especially 15°C – S_A 20 featured no DEGs coding for photosynthesis compared to the control group (8°C – S_A 30). Similarly, 15°C – S_A 20 resulted in the lowest number of DEGs among the low salinity treatments. Both results suggest that 15°C could reduce the negative effect of hyposaline on the transcriptomic level for *S. latissima* after a short-term exposure. It might be that moderately increased temperature can increase the enzymatic reactions of photosynthesis and reduce the formation of ROS, since the unbalanced electron chain at low temperature between photosynthetic primary and enzymatic reactions may result in an increased transfer of electrons to O_2, eventually promoting the generation of ROS (Polle 1996, Hurd et al. 2014). This oxidative stress leads to chronic photoinhibition, bleaching of photosynthetic pigments and peroxidation of membrane lipids (Aro et al. 1993, Osmond 1994). These were also reflected by the low values of F_v/F_m at 0°C. Low temperature (0°C) exclusively repressed the gene encoding chloroplastic PGK, which was also downregulated in the brown seaweed *Desmarestia anceps* under low temperature and high light stress (Iñiguez et al. 2017).

PGK in the chloroplast is an important enzyme for producing 1,3-bisphosphoglycerate in the photosynthetic carbon metabolism (Joshi et al. 2016).

General stress responses

In our study genes encoding GSTs were induced at 0°C and repressed at 15°C. GSTs are a detoxification enzyme family, catalyzing the conjugation of the tripeptide glutathione with electrophilic substrates (Xu et al. 2015). They have several functions such as xenobiotic detoxification, non-enzymatic carriers (ligandins), modulators of signaling processes and metabolism of endogenous compounds (de Franco et al. 2008). Heinrich et al. (2012b) observed an induction of GSTs in *Saccharina latissima* in response to high temperatures (17°C). Furthermore, GSTs were induced under different environmental stresses (Collen et al. 2006, Cosse et al. 2009), which might be partly due to their different functions. In addition, 0°C – S_A 20 caused induction of one DEG encoding mitochondrial AOX. AOX can avoid or alleviate the generation of ROS by diverting electrons flowing to reduce O_2 to water (Mittler 2002). In 0°C – S_A 20 the gene encoding peptide methionine sulfoxide reductase MsrA showed a significantly higher abundance. This enzyme is involved in the repair of oxidized proteins by catalyzing the reduction of methionine sulfoxide to methionine (Hansel et al. 2002). It is believed that MsrA may be distributed in the areas where the concentration of ROS is high (Hoshi and Heinemann 2001). The induction of GSTs at 0°C, MsrA and AOX in 0°C – S_A 20 suggests that low temperature impairs the physiological state of sporophytes that respond by avoiding production of ROS and increasing antioxidant activity.

Several DEGs encoding vBPO were upregulated in 8°C – S_A 20, while repressed at 15°C within S_A 20 and S_A 30. Two studies showed that specific bromoperoxidase isoforms were upregulated after protoplast isolation in *S. latissima* and *L. digitata* (Jordan et al. 1991, Roeder et al. 2005). Furthermore, *G. changii* also induced the gene coding for vBPO in both hyper- and hypoosmotic stress (Teo et al. 2009). In general, haloperoxidases are considered as critical enzymes for oxidative stress management in kelp (Bischof and Rautenberger 2012). One function of vBPO in marine algae is to scavenge the harmful H_2O_2 by producing halogenated compounds (Pedersén et al. 1996, Ohsawa et al. 2001). Another possible role of vBPOs is correlated with the oxidative cross-linking of alginates and polyphenols, strengthening the cell wall and/or algal adhesion (Berglin et al. 2004). The enhancement of vBPO activities in cell-wall regenerating protoplasts of *S. latissima* suggested that they could also take part in cell wall assembly (Jordan et al. 1991). This

might be the case in our study, as sporophytes of *S. latissima* may exhibit cellular adjustments and change cell turgor with low salinity, in addition to regulate the ion and metabolite concentrations to protect themselves from salinity changes. Similarly, cell walls of estuarine *Ulva intestinalis* were thinner and more flexible than those of marine and rock-pool specimens of the same species, enabling them to swell with the influx of water in low salinity environments (Edwards et al. 1987). When it comes to the downregulation of vBPOs in 15°C – S_A 20, 15°C could alleviate the production of ROS induced by low salinity compared to 8°C, because the optimal temperature for the sporophyte growth of *S. latissima* is between 10 and 15°C (Fortes and Lüning 1980). This is also in agreement with our physiological data, showing that sporophytes grew best at 15°C. In general, the vanadium-dependent haloperoxidases, as Cosse et al. (2007) suggested, are involved in defense responses of macroalgae with three putative biological roles, which are oxidative detoxification, cell wall strengthening and chemical defense.

Apart from the upregulation of stress-related DEGs in 0°C and vBPOs in 8°C – S_A 20, the typical ROS scavenging enzymes (i.e. superoxide dismutase, ascorbate peroxidase and catalase) were not significantly upregulated, however, featured downregulation in 8°C – S_A 20. The reason might be that several genes encoding ROS scavenging enzymes showed a high level of expression in the control, e.g. superoxide dismutase (Mn)/(Fe)/(Cu-Zn). These three groups of superoxide dismutase, distributed in different compartments of cells, are crucial for ROS scavenging and constitute the first line of defense against ROS (Alscher et al. 2002). The constitutively high expression of ROS scavengers may explain why these genes were not differentially expressed in the different treatments.

Additional to the increase of ROS scavenging-related metabolism, kelps tend to increase the expression of specific protective proteins (Bischof and Rautenberger 2012). HSPs and other chaperones play important roles in keeping cellular homeostasis, functioning in protein folding, stabilizing proteins and membranes, and eliminating non-native aggregations under abiotic stresses (Wang et al. 2004). Previous studies have shown that low temperature, osmotic-, salinity-, oxidative- and other abiotic stresses can induce gene expression and synthesis of HSPs/chaperones (Swindell et al. 2007, Al-Whaibi 2011). However, only two DEGs encoding HSPs were downregulated in our study. One reason might be that sporophytes were already temperature pre-acclimated before conducting the salinity treatments, another reason for the limited regulation of HSPs in the treatments might be their constitutive high

expression in the control (8°C – S$_A$ 30). This constitutive expression of stress-related genes may increase the tolerance of *S. latissima* to different abiotic stresses and helps *S. latissima* to acclimate to the new environment in short time.

Osmoregulation

Osmotic acclimation is a fundamental mechanism of algae in salinity stress to keep up the stability of the intracellular milieu (homeostasis) and maintain its functional state (Kirst 1990, Karsten 2012). When algae are under hyposaline stress, water influx to the cell occurs because the water potential changes (Russell 1987). This is phase I of osmotic acclimation, lasting from minutes to hours (Zimmermann and Steudle 1979). Phase II of osmotic acclimation consists in the adjustment of the intracellular osmolyte concentrations to a new steady state by regulating the inorganic ions (fast) and organic osmolytes (slow), which is under direct cellular metabolic control (Karsten 2012). Even though algae are supposed to import ions by ion-selective carriers under hyposalinity, we only found a few of DEGs encoding ion transporters down-regulated under S$_A$ 20 treatment (e.g. potassium channel in 0°C – S$_A$ 20). However, several DEGs coding for ABC transporters showed various expression patterns in response to the treatments. 0°C – S$_A$ 20 and 8°C – S$_A$ 20 promoted both up and downregulation, while 15°C only resulted in down-regulated DEGs encoding ABC transporters. Such expression patterns have also been described for the euryhaline alga, *Prymnesium parvum* under hypersaline stress (Talarski et al. 2016). The functions of ABC transporters are extensive and include transporting mineral ions, lipids and peptides (Rea 2007). Hence, we infer that ABC transporters play an important role in the osmotic acclimation of algae.

Considering organic osmolytes, mannitol is widely believed to be a characteristic osmolyte for most Phaeophyceae (Davison and Reed 1985, Reed et al. 1985, Kirst 1990). The mannitol-1-*P* dehydrogenase gene was downregulated in *E. siliculosus* under hyposaline stress (Dittami et al. 2009), but not in *S. latissima* in our study. In contrast, several DEGs encoding oxygen-dependent choline dehydrogenase were downregulated in 0°C – S$_A$ 20 and 8°C – S$_A$ 20, as well as in 0°C – S$_A$ 30. Oxygen-dependent choline dehydrogenase is involved in the biosynthesis of the osmoprotectant glycine betaine (Lilius et al. 1996). Glycine betaine is an important compatible osmolyte that accumulates in plants, animals and bacteria under abiotic stresses such as drought and salinity (Yancey 1994, Gorham 1995). Biosynthesis of glycine betaine via choline dehydrogenase also occurred in the diatom *Thalassiosira pseudonana* under hypersaline

conditions (Kageyama et al. 2018). Hence, we infer that *S. latissima* tends to repress the genes encoding for choline dehydrogenase, which might ultimately lead to the decrease of glycine betaine, to reduce the osmotic pressure under hyposaline stress. Apart from the role as osmoprotectant, glycine betaine can protect organisms from low temperature by maintaining the membrane integrity (Zhao et al. 1992). Several studies have found that glycine betaine was accumulated in response to low temperatures (Naidu et al. 1991, Jin et al. 2015). However, 0°C – S$_A$ 30 in our study repressed the expression of the gene encoding the oxygen-dependent choline dehydrogenase, which prompts for further investigation.

Ecological implications

Previous studies have shown that the responses of seaweeds to abiotic stress varies over different developmental and life-history stages (Hanelt et al. 1997, Fredersdorf et al. 2009). In general, young sporophytes are more sensitive than old sporophytes, as they have less cellular layers to protect their chloroplasts and other organelles from the damage of stressors as discussed by Hanelt et al. (1997). In our study, we used young sporophytes with an average length of about 12 cm. The higher vulnerability of this younger stage will influence the abundance of the adult population and thus is a crucial piece in the evaluation of the effects of climate change in the Arctic population of *S. latissima*.

Temperature is the main abiotic factor directly controlling geographic boundaries of seaweeds, thus temperature changes can affect the geographical distribution of seaweeds strongly (van den Hoek 1982, Lüning et al. 1990, Eggert 2012). As exhibited by a previous study, Arctic *S. latissima* improved its F_v/F_m and growth in a warming scenario (Iñiguez et al. 2016). Our results also showed that increased temperature has positive effects on growth, F_v/F_m and pigment contents of *S. latissima*. It indicates that *S. latissima* displays a broad physiological tolerance to temperature and still exhibits optimal growth at the ambient temperature regime of its central distribution. However, a higher level of transcriptomic adjustment is required at the higher temperature tested (15°C) than at low temperatures (0°C), a temperature which is nearer to conditions experienced in the field of an Arctic strain. We infer that the relatively short cold-water history in the Arctic can explain the tolerance of *S. latissima* to temperatures far above those typical for Polar regions (Hurd et al. 2014). However, the changes in gene expression observed, might result in energy costs involved in other physiological parameters not studied here.

Our results support the prediction of Müller et al. (2009) that *S. latissima* may occupy even higher latitudes

up to the North of Spitsbergen by 2080–2099. As reported by Müller et al. (2009), the sea surface temperatures will rise by 3–4°C by the end of this century, the higher growth at 8°C and 15°C in our study indicates that the Arctic *S. latissima* could benefit from global warming and might increase its biomass in the future. However, increasing summer temperatures already had a negative effect in *S. latissima* at lower latitudes. Especially, a large-scale decline of *S. latissima* has been observed along the coast of southern Norway (Moy and Christie 2012). Although the reason of such large loss is not clear, the authors suggested that eutrophication (nutrient and particle pollution) and climate change (increased temperature) are two main drivers. Such phenomenon also highlights that the interaction of temperature with other factors is important to consider when evaluating trends of kelp distribution under climate change scenarios.

Among others, temperature increase will enhance terrestrial and glacial run-off, with the potential to alter salinity regimes in Arctic fjord systems (Hanelt et al. 2001). Apparently, S_A 20 in our study did not provoke an acute decrease in physiological performance of *S. latissima*. But under prolonged exposure to S_A 20 the growth of *S. latissima* was reduced, as the fresh weight in S_A 20 was significantly lower than at S_A 30 on day 18. Thus we infer that the energy cost for successful acclimation might exceed the energy available, limiting the ability of *S. latissima* to acclimate to long-term hyposaline exposure. Since the growth of *S. latissima* at 0°C was lower than at 8 or 15°C, the reduced growth rates induced by S_A 20 might exacerbate the negative effects on the physiological state of *S. latissima* at low temperature, compared to the decreased growth rates at higher temperature. The severe pigment bleaching in 0°C – S_A 20 on day 18 also suggests that *S. latissima* might be on the brink of death under prolonged hyposaline conditions.

Moderate salinity drop (S_A 30 to S_A 20) is one targeted in our study, which might not induce an acute adverse effect on *S. latissima* according to our results, but still leads to transcriptomic reorganization. This re-allocation of resources to prevent damage might ultimately affect the growth of *S. latissima* in the long run. Elevated temperature may contribute to the amelioration of the adverse effects of hyposalinity in *S. latissima*. Hence the potentially inhibiting function of hyposalinity to *S. latissima* should be considered in the network of all environmental factors.

Author contributions

H.L., C.M., S.H., K.B., I.B. and K.V. designed the experiment. H.L. conducted the experiment, carried out the physiological measurements and RNA extractions, analyzed the data and wrote the manuscript. C.M. also conducted the experiment and carried out the physiological measurements. S.H. supervised the RNA extraction and transcriptomic data interpretation. I.B. provided the algae cultures and the laboratory facilities for algae cultivation and co-designed the experiments. E.C. assembled the de novo transcriptome and L.H. performed the bioinformatics analysis of RNA-Seq data. G.G. carried out the RNA sequencing. K.B., S.H., I.B. and K.V. supervised the project and contributed to data interpretation and discussion. All authors reviewed, corrected and approved the final version of the manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Results of the repeated measures ANOVA for effects of temperature on fresh weights during 7 days of temperature pre-acclimation.

Table S2. Results of the repeated measures ANOVA for effects of temperature and salinity on fresh weights during 11 days of salinity treatment within three temperatures.

Table S3. Results of the repeated measures ANOVA for effects of temperature on F_v/F_m during 7 days of temperature pre-acclimation.

Table S4. Results of the repeated measures ANOVA for effects of temperature and salinity on F_v/F_m during 11 days of salinity treatment within three temperatures.

Table S5. Results of the two-way ANOVA for effects of temperature and salinity on pigments measured on day 8.

Table S6. Results of the two-way ANOVA for effects of temperature and salinity on pigments measured on day 18.

Table S7. Transcripts per million (TPM) counts of Trinity genes in the control treatment encoding photosynthesis and photosynthetic components, heat shock proteins, ROS scavenging system and potential osmolytes.

Fig. S1. Photograph of the spot-labeled sporophytes under different treatments on the last day of experiment (day 18).

Fig. S2. Relative growth rates of fresh weights of *Saccharina latissima* after 7 days of temperature pre-acclimation and 11 days of temperature and salinity treatment.

Fig. S3. Pigment contents and de-epoxidation states of xanthophyll cycle (DPS) of *Saccharina latissima* after exposure to two salinity conditions (S_A 20, 30) within three temperatures (0, 8, 15°C) on day 18.

Fig. S4. Functional categories derived from enriched GO terms of down-regulated genes of treatment groups compared to the control (8°C – S_A 30).

Table S8. Full list of differentially expressed genes against the control treatment (8°C – S_A 30).

Table S1. Results of the repeated measures ANOVA for effects of temperature and time on fresh weights measured during 7 days of temperature pre-acclimation for *Saccharina latissima*. Statistically significant values are indicated by asterisks ($p \leq 0.05$).

Variable	Source	*df*	MS	*F* ratio	*p* value
Fresh weight	Within-subjects effects				
	Time	1.451	146930.909	52.056	< 0.001*
	Time × Temperature	2.902	5165.85	1.83	0.173
	Between-subjects effects				
	Temperature	2	11944.191	3.033	0.078
	Error	15	3938.398		

Table S2. Results of the repeated measures ANOVA for effects of temperature, salinity and time on fresh weights measured during 11 days of salinity treatment within three temperatures for *Saccharina latissima*. Statistically significant values are indicated by asterisks ($p \leq 0.05$). The homogeneity of variance was violated for fresh weights on day 7.

Variable	Source	*df*	MS	*F* ratio	*p* value
Fresh weight	Within-subjects effects				
	Time	1.419	536280.964	51.743	< 0.001*
	Time × Temperature	2.839	63532.758	6.13	0.006*
	Time × Salinity	1.419	77153.498	7.444	0.009*
	Time × Temperature × Salinity	2.839	24108.379	2.326	0.114
	Error	17.032	10364.419		
	Between-subjects effects				
	Temperature	2	388249.643	8.075	0.006*
	Salinity	1	192541.13	4.005	0.069
	Temperature × Salinity	2	11296.138	0.235	0.794
	Error	12	48078.341		

Table S3. Results of the repeated measures ANOVA for effects of temperature on F_v/F_m during 7 days of temperature pre-acclimation for *Saccharina latissima*. Statistically significant values are indicated by asterisks ($p \leq 0.05$).

Variable	Source	*df*	MS	*F* ratio	*p* value
F_v/F_m	Within-subjects effects				
	Time	1.371	0.015	192.542	< 0.001*
	Time × Temperature	2.742	0.004	56.025	< 0.001*
	Between-subjects effects				
	Temperature	2	0.005	9.894	0.003*
	Error	12	0.001		

Table S4. Results of the repeated measures ANOVA for effects of temperature and salinity on F_v/F_m during 11 days of salinity treatment for *Saccharina latissima*. Statistically significant values are indicated by asterisks ($p \leq 0.05$). The homogeneity of variance was violated for F_v/F_m on day 14.

Variable	Source	*df*	MS	*F* ratio	*p* value
F_v/F_m	Within-subjects effects				
	Time	2.598	0.002	4.373	0.010*
	Time × Temperature	5.196	0.002	3.736	0.005*
	Time × Salinity	2.598	0.005	10.922	< 0.001*
	Time × Temperature × Salinity	5.196	0.003	5.846	< 0.001*
	Between-subjects effects				
	Temperature	2	0.035	170.640	< 0.001*
	Salinity	1	0.001	3.553	0.072
	Temperature × Salinity	2	<0.001	0.543	0.588
	Error	24	<0.001		

Table S5. Results of the two-way ANOVA for effects of temperature and salinity on pigments measured on day 8 for *Saccharina latissima.* Statistically significant values are indicated by asterisks ($p \leq$ 0.05). Acc and DPS were analysed by non-parametric tests as they had abnormal distributions.

Variable	Factor	*df*	MS	*F* ratio	*p* value
Chlorophyll *a*	Temperature	2	0.522	3.503	0.047
	Salinity	1	0.567	3.805	0.063
	Temperature × Salinity	2	0.128	0.859	0.437
	Error	23	0.149		
VAZ	Temperature	2	0.012	8.019	0.002*
	Salinity	1	0.008	5.332	0.03*
	Temperature × Salinity	2	< 0.001	0.034	0.967
	Error	23	0.001		
Acc	Temperature	Kruskal-Wallis Test			0.001*
	Salinity	Mann-Whitney *U* Test			0.290
DPS	Temperature	Kruskal-Wallis Test			0.072
	Salinity	Mann-Whitney *U* Test			0.78

Table S6. Results of the two-way ANOVA for effects of temperature and salinity on pigments measured on day 18 for *Saccharina latissima.* Statistically significant values are indicated by asterisks ($p \leq$ 0.05). Chlorophyll *a* was analysed by non-parametric tests as it had abnormal distributions.

Variable	Factor	*df*	MS	*F* ratio	*p* value
Acc	Temperature	2	0.683	5.187	0.013*
	Salinity	1	0.165	1.253	0.274
	Temperature × Salinity	2	0.508	3.859	0.035*
	Error	24	0.132		
VAZ	Temperature	2	0.001	1.078	0.356
	Salinity	1	0.012	11.175	0.003*
	Temperature × Salinity	2	0.001	0.743	0.486
	Error	24	0.001		
DPS	Temperature	2	0.009	41.098	< 0.001*
	Salinity	1	0.004	17.247	< 0.001*
	Temperature × Salinity	2	0.001	2.909	0.074
	Error	24	< 0.001		
Chlorophyll *a*	Temperature	Kruskal-Wallis Test			0.525
	Salinity	Mann-Whitney *U* Test			0.174

Table S7. Transcripts per million (TPM) counts of Trinity genes (above the average TPM value: 22) in the control treatment (8°C - S_A 30) encoding photosynthesis and photosynthetic components, heat shock proteins, ROS scavenging system and potential osmolytes. Most of them were constitutively expressed in all treatments.

Trinity gene name	Putative gene product	Annotation e-Value	TPM
Photosynthesis and photosynthetic components			
TRINITY_DN30544_c6_g1	Fucoxanthin-chlorophyll *a-c* binding protein F	8.3e-34	16570
TRINITY_DN30544_c6_g5	Fucoxanthin-chlorophyll *a-c* binding protein B	6.8e-56	5236
TRINITY_DN29722_c7_g2	Fucoxanthin-chlorophyll *a-c* binding protein D	1.9e-40	4054
TRINITY_DN29200_c1_g4	Fucoxanthin-chlorophyll *a-c* binding protein A	3.1e-33	3147
TRINITY_DN28592_c7_g5	Fucoxanthin-chlorophyll *a-c* binding protein	2.5e-19	2150
TRINITY_DN29729_c0_g1	Fucoxanthin-chlorophyll *a-c* binding protein B	1.7e-70	1882
TRINITY_DN28702_c0_g1	Photosystem II 12 kDa extrinsic protein	3.3e-28	1876
TRINITY_DN29000_c6_g1	Glyceraldehyde-3-phosphate dehydrogenase	1.1e-17	1824
TRINITY_DN28961_c6_g1	Chlorophyll *a-b* binding protein 2	1.1e-12	1712
TRINITY_DN28242_c3_g2	Light-harvesting complex I LH38 proteins	4.3e-10	1697
TRINITY_DN28609_c9_g1	Chlorophyll *a-b* binding protein L1818	9.6e-16	1649
TRINITY_DN28988_c7_g1	Phosphoglycerate kinase	2.6e-150	1491
TRINITY_DN28856_c4_g1	Chlorophyll *a-b* binding protein 1B-21	3.8e-09	1439
TRINITY_DN29200_c1_g5	Fucoxanthin-chlorophyll *a-c* binding protein E	3.7e-98	1310
TRINITY_DN28777_c1_g5	Light-harvesting complex I LH38 proteins	9.9e-14	1248
TRINITY_DN29649_c4_g2	Ferredoxin--NADP reductase	3.8e-105	1169
TRINITY_DN29722_c7_g6	Fucoxanthin-chlorophyll *a-c* binding protein C	8.3e-06	1014
TRINITY_DN29729_c0_g3	Fucoxanthin-chlorophyll *a-c* binding protein F	7.3e-27	975
TRINITY_DN29881_c3_g3	Oxygen-evolving enhancer protein 1	1.3e-40	944
TRINITY_DN28601_c5_g1	Photosystem I chlorophyll *a/b*-binding protein 5	1.4e-16	927
TRINITY_DN29200_c1_g2	Fucoxanthin-chlorophyll *a-c* binding protein B	4.1e-77	913
TRINITY_DN28012_c3_g2	Glucose-6-phosphate/phosphate translocator 2	1e-57	904
TRINITY_DN29604_c2_g2	Magnesium-chelatase subunit ChlH	0	691
TRINITY_DN29909_c5_g1	Sedoheptulose-1,7-bisphosphatase	3.1e-26	668
TRINITY_DN29217_c3_g1	Fructose-1,6-bisphosphatase 1	7.1e-96	663
TRINITY_DN26828_c0_g1	Glucose-6-phosphate/phosphate translocator 2	2.4e-56	648
TRINITY_DN27134_c6_g1	Chlorophyll *a-b* binding protein 1B-21	1.5e-11	613
TRINITY_DN27779_c10_g1	Phosphoribulokinase	1.6e-141	606
TRINITY_DN28450_c6_g5	Cytochrome b6-f complex iron-sulfur subunit	5.9e-61	526
TRINITY_DN27082_c10_g4	Chlorophyll *a-b* binding protein L1818	1e-06	505
TRINITY_DN29151_c2_g1	Geranylgeranyl diphosphate reductase	2.4e-92	489
TRINITY_DN26561_c13_g1	Chlorophyll *a-b* binding protein CP29.2	1e-14	456
TRINITY_DN26390_c14_g1	Fucoxanthin-chlorophyll *a-c* binding protein D	4.4e-07	398
TRINITY_DN28761_c0_g1	Chlorophyll *a-b* binding protein L1818	3e-11	322
TRINITY_DN30607_c5_g5	Chlorophyll *a-b* binding protein L1818	1.4e-12	273
TRINITY_DN28988_c5_g2	Phosphoglycerate kinase	5.3e-65	258
TRINITY_DN30607_c4_g1	Chlorophyll *a-b* binding protein L1818	2.1e-10	253
TRINITY_DN27874_c8_g1	Protein thylakoid formation 1	3.2e-22	237
TRINITY_DN27117_c5_g2	ATP-dependent zinc metalloprotease FTSH 5	3.1e-114	190
TRINITY_DN30235_c1_g4	Chlorophyll *a-b* binding protein L1818	3e-25	179
TRINITY_DN28988_c5_g1	Phosphoglycerate kinase	6.1e-25	178
TRINITY_DN30327_c7_g2	Chlorophyll *a-b* binding protein L1818	3.3e-10	170
TRINITY_DN26697_c7_g1	Photosystem II repair protein PSB27-H1	3.1e-14	151
TRINITY_DN27421_c2_g2	Magnesium protoporphyrin IX methyltransferase	9.8e-69	107
TRINITY_DN27928_c3_g1	Zeaxanthin epoxidase	1.5e-69	95
TRINITY_DN26767_c6_g1	Ferredoxin-1	1.1e-08	74
TRINITY_DN20017_c0_g1	Inner membrane ALBINO3-like protein 2	3.1e-44	66
TRINITY_DN29194_c2_g1	Light-harvesting complex-like protein OHP2	8.4e-07	53
TRINITY_DN28229_c8_g2	Protoporphyrinogen oxidase 1	1.5e-47	51
TRINITY_DN29519_c0_g1	Violaxanthin de-epoxidase	5.5e-82	44

TRINITY_DN30265_c9_g1	Chlorophyll synthase	2.9e-122	28
TRINITY_DN27688_c8_g1	Fructose-1,6-bisphosphatase 1	1e-35	25
TRINITY_DN27115_c9_g2	Violaxanthin de-epoxidase	4.1e-22	24
TRINITY_DN29561_c9_g1	Fucoxanthin-chlorophyll *a-c* binding protein	1.9e-28	22
Heat shock proteins			
TRINITY_DN30375_c6_g1	Heat shock protein 90-1	6.5e-113	823
TRINITY_DN30464_c1_g1	Heat shock cognate 70 kDa protein	2.3e-25	756
TRINITY_DN27078_c0_g1	Heat shock 70 kDa protein 5	2e-24	724
TRINITY_DN28363_c10_g1	78 kDa glucose-regulated protein	4.9e-83	550
TRINITY_DN27875_c2_g2	Heat shock 70 kDa protein 5	2.1e-137	254
TRINITY_DN28998_c2_g2	Probable assembly chaperone of rpl4	4e-21	82
TRINITY_DN27183_c5_g2	Chaperone protein DnaK	2.7e-231	80
TRINITY_DN27762_c7_g1	Heat shock protein 90-5	6.7e-44	72
TRINITY_DN60391_c0_g1	Hsp90 co-chaperone Cdc37	1.1e-35	49
TRINITY_DN26569_c2_g4	Heat shock 70 kDa protein 1B	9.6e-64	46
TRINITY_DN30250_c0_g1	Mitochondrial chaperone BCS1	4.8e-11	35
TRINITY_DN27426_c10_g1	Mitochondrial chaperone BCS1	1.2e-10	30
TRINITY_DN26698_c9_g3	Heat shock protein sti1 homolog	1.8e-16	27
TRINITY_DN27313_c8_g1	Activator of 90 kDa heat shock protein ATPase homolog 2	2.8e-09	26
TRINITY_DN26054_c0_g1	78 kDa glucose-regulated protein	2e-85	22
TRINITY_DN26904_c6_g9	Chaperone protein DnaJ	2.3e-18	22
ROS scavenging system			
TRINITY_DN30059_c1_g4	Vanadium-dependent bromoperoxidase	3.5e-11	3149
TRINITY_DN27187_c7_g1	Vanadium-dependent bromoperoxidase	2.6e-25	1165
TRINITY_DN30022_c1_g1	Vanadium-dependent bromoperoxidase	1.4e-56	789
TRINITY_DN29878_c9_g2	Vanadium-dependent bromoperoxidase	1.6e-79	611
TRINITY_DN26334_c3_g2	Superoxide dismutase (Mn)	1.9e-35	545
TRINITY_DN30345_c4_g3	Vanadium-dependent bromoperoxidase	1.8e-19	265
TRINITY_DN29226_c7_g3	Putative heme-binding peroxidase	7e-72	217
TRINITY_DN27930_c7_g2	Glutaredoxin	5.5e-09	213
TRINITY_DN26569_c2_g6	Superoxide dismutase (Fe)	4.5e-08	201
TRINITY_DN28908_c2_g1	Vanadium-dependent bromoperoxidase	3.2e-36	200
TRINITY_DN30390_c2_g1	Vanadium-dependent bromoperoxidase	2.5e-90	189
TRINITY_DN29181_c1_g1	Vanadium-dependent bromoperoxidase	4.1e-58	165
TRINITY_DN29577_c3_g2	Probable L-ascorbate peroxidase 6	3.4e-73	153
TRINITY_DN26836_c8_g7	Superoxide dismutase (Cu-Zn)	3.8e-31	149
TRINITY_DN29164_c10_g6	Probable L-ascorbate peroxidase 8	2.6e-11	135
TRINITY_DN26999_c7_g3	Cytochrome c peroxidase	2.1e-58	132
TRINITY_DN30053_c6_g5	Vanadium-dependent bromoperoxidase	2.5e-73	105
TRINITY_DN28139_c1_g3	Monothiol glutaredoxin-S7	4.4e-31	97
TRINITY_DN27538_c12_g4	L-ascorbate peroxidase T	8.2e-39	92
TRINITY_DN28908_c2_g4	Vanadium-dependent bromoperoxidase	1.4e-10	92
TRINITY_DN29181_c1_g2	Vanadium-dependent bromoperoxidase	1.5e-09	85
TRINITY_DN30022_c1_g10	Vanadium-dependent bromoperoxidase	1e-38	84
TRINITY_DN27187_c6_g2	Vanadium-dependent bromoperoxidase	4.7e-91	82
TRINITY_DN27680_c4_g1	Catalase	1.5e-176	72
TRINITY_DN29065_c7_g1	Vanadium-dependent bromoperoxidase	3e-08	68
TRINITY_DN26517_c11_g3	Superoxide dismutase (Mn)	4.1e-70	64
TRINITY_DN30390_c2_g14	Vanadium-dependent bromoperoxidase	8.3e-12	55
TRINITY_DN26543_c4_g1	Thioredoxin	5.8e-16	55
TRINITY_DN25903_c0_g1	Peroxiredoxin Q	1.5e-35	47
TRINITY_DN26476_c10_g1	Glutaredoxin arsenate reductase	3.6e-39	45
TRINITY_DN28091_c0_g2	Thioredoxin-like protein HCF164	1.7e-24	43
TRINITY_DN39666_c0_g1	Thioredoxin-2	2e-10	38
TRINITY_DN27355_c7_g1	Monothiol glutaredoxin-S15	3.1e-10	36
TRINITY_DN26522_c10_g1	Thioredoxin H-type	1.9e-20	33
TRINITY_DN26569_c2_g1	Superoxide dismutase (Fe)	1.4e-57	29

|			
TRINITY_DN27832_c4_g1	Glutaredoxin 2	6.1e-28	27
TRINITY_DN28040_c2_g1	Thioredoxin	7e-18	22
Potential osmolytes			
TRINITY_DN25054_c0_g1	Oxygen-dependent choline dehydrogenase	2.7e-69	103
TRINITY_DN29245_c5_g1	Mannitol 2-dehydrogenase	1.5e-93	60
TRINITY_DN29689_c1_g1	Oxygen-dependent choline dehydrogenase	9.8e-29	60
TRINITY_DN29689_c1_g2	Oxygen-dependent choline dehydrogenase	2.9e-13	24
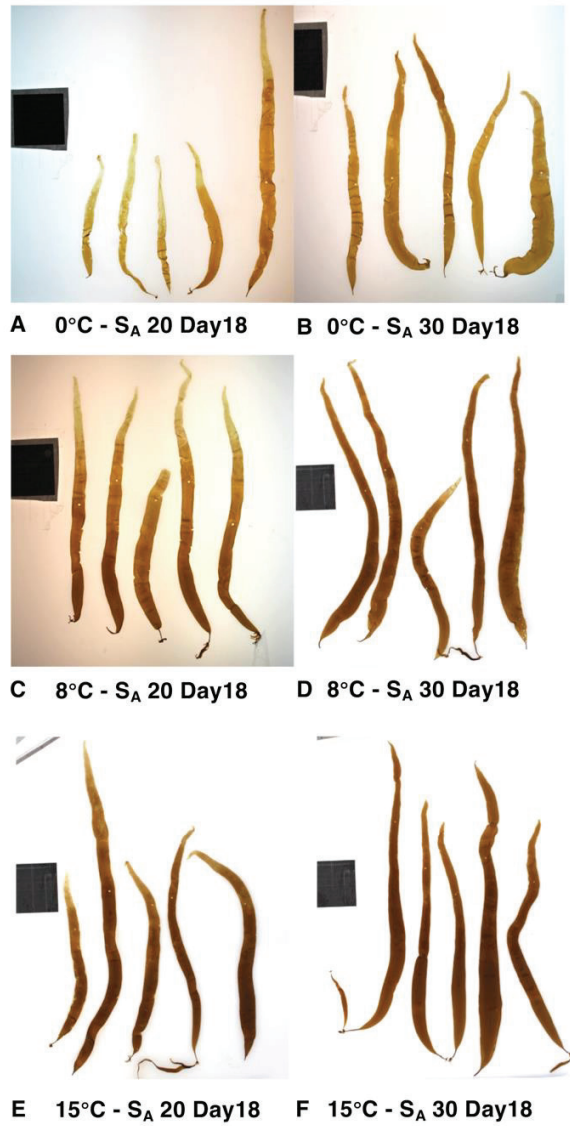
Figure S1. Photograph of the spot-labeled sporophytes at 0°C - S_A 20 (A), 0°C - S_A 30 (B), 8°C - S_A 20 (C), 8°C - S_A 30 (D), 15°C - S_A 20 (E) and 15°C - S_A 30 (F) on the last day of experiment (day 18).
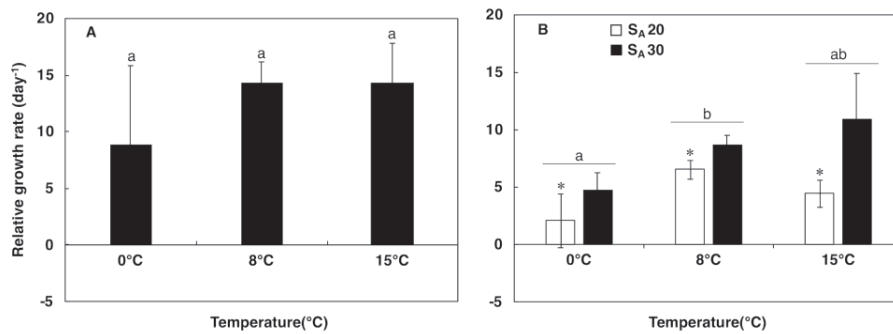
Figure S2. Relative growth rates (day^{-1}, mean ± standard deviation; n = 3) of fresh weights of *Saccharina latissima* after 7 days of temperature pre-acclimation (from day 0 to day 7; A) and 11 days of temperature and salinity treatment (from day 7 to day 18; B). Significant differences between temperature and salinity are shown by alphabetic characters and asterisk respectively ($p \leq 0.05$).
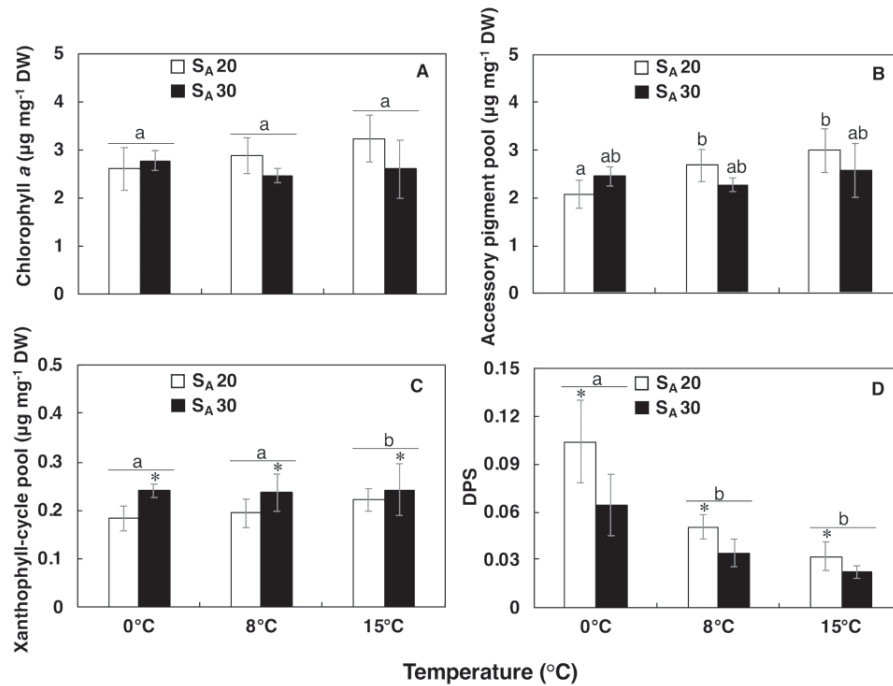
Figure S3. Chlorophyll *a* content (A), accessory pigment pool content (Acc; B), xanthophyll-cycle pool content (VAZ; C) (µg mg^{-1} DW; mean ± standard deviation; n = 5) and the de-epoxidation states of xanthophyll cycle (DPS; D) (mean ± standard deviation; n = 5) of *Saccharina latissima* after exposure to two salinity conditions (S$_A$ 20, 30) within three temperatures (0, 8, 15°C) on day 18. Significant differences between temperature and salinity are shown by alphabetic characters and asterisk respectively ($p \leq 0.05$). The difference of Acc between each treatment was shown by alphabetic characters since the interaction of temperature × salinity was significant.

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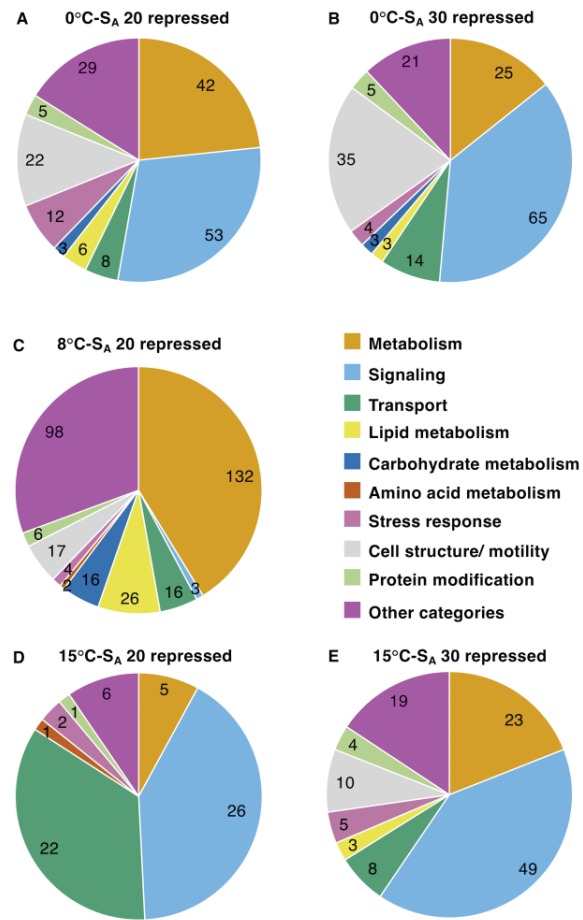
Figure S4. Functional categories derived from enriched GO terms of down-regulated genes of treatment 0°C - S_A 20 (A), 0°C - S_A 30 (B), 8°C - S_A 20 (C), 15°C - S_A 20 (D) and 15°C - S_A 30 (E) compared to the control (8°C - S_A 30): Classification after EGAD2GO using cateGOrizer.

4   Is geographical variation driving the transcriptomic responses to multiple stressors in the kelp *Saccharina latissima*?

Is geographical variation driving the transcriptomic responses to multiple stressors in the kelp *Saccharina latissima*?

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Abstract

Background: Kelps (Laminariales, Phaeophyceae) are brown macroalgae of utmost ecological, and increasingly economic, importance on temperate to polar rocky shores. Omics approaches in brown algae are still scarce and knowledge of their acclimation mechanisms to the changing conditions experienced in coastal environments can benefit from the application of RNA-sequencing.

Despite evidence of ecotypic differentiation, transcriptomic responses from distinct geographical locations have, to our knowledge, never been studied in the sugar kelp *Saccharina latissima* so far.

Results: In this study we investigated gene expression responses using RNA-sequencing of *S. latissima* from environments with contrasting temperature and salinity conditions – Roscoff, in temperate eastern Atlantic, and Spitsbergen in the Arctic. Juvenile sporophytes derived from uniparental stock cultures from both locations were pre-cultivated at 8 °C and S_A 30. Sporophytes acclimated to 0 °C, 8 °C and 15 °C were exposed to a low salinity treatment (S_A 20) for 24 h. Hyposalinity had a greater impact at the transcriptomic level than the temperature alone, and its effects were modulated by temperature. Namely, photosynthesis and pigment synthesis were extensively repressed by low salinity at low temperatures. Although some responses were shared among sporophytes from the different sites, marked differences were revealed by principal component analysis, differential expression and GO enrichment. The interaction between low temperature and low salinity drove the largest changes in gene expression in sporophytes from Roscoff while specimens from Spitsbergen required more metabolic adjustment at higher temperatures. Moreover, genes related to cell wall adjustment were differentially expressed between Spitsbergen and Roscoff control samples.

Conclusions: Our study reveals interactive effects of temperature and salinity on transcriptomic profiles in *S. latissima*. Moreover, our data suggest that under identical culture conditions sporophytes from different locations diverge in their transcriptomic responses. This is probably connected to variations in temperature and salinity in their respective environment of origin. The current transcriptomic results support the plastic response pattern in sugar kelp which is a species with several reported ecotypes. Our data provide the baseline for a better understanding of the underlying processes of physiological plasticity and may help in the future to identify strains adapted to specific environments and its genetic control.

Keywords: Gene expression, Brown algae, Temperature stress, Salinity stress, Geographical variation, RNA-seq

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Background

Kelps are brown macroalgae of the order Laminariales that dominate the rocky benthic ecosystems in temperate to polar regions. They play an important ecological role by contributing greatly to the primary production in coastal systems and by providing shelter and nursery for many animal species [1, 2]. Furthermore, they hold economic value, namely for food, feed, and raw material for pharmaceutical industries and are of growing interest for the aquaculture sector [3–5].

Saccharina latissima (L.) C.E. Lane, C. Mayes, Druehl, et G. W. Saunders is a species of Laminariales that has a wide latitudinal range in the Northern hemisphere from polar to temperate waters [6]. In the eastern Atlantic, it is distributed from the Arctic (> 80°N) to the north of Portugal (41.5°N) [7]. This suggests that the species has a high potential to adapt to variations in temperature and irradiance but the level of adaptation along latitudinal gradients is not clear [8, 9]. The optimum growth range for *S. latissima* is between 10 °C and 15 °C; above 20 °C the mortality rate is high [10, 11]. Concomitantly, *S. latissima* exhibits optimum growth at absolute salinities (S_A) between 23 and 31, shows a strong reduction around S_A 13 and a high mortality below S_A 8 [12, 13].

The physiological tolerance to several biotic and abiotic stress factors is well-known in *S. latissima* e.g. [1, 8–12, 14]; however, the underlying molecular bases remain poorly understood; respective studies have been limited to temperature, irradiance, and UV radiation [15–17].

The variable environments along its distributional range have created ecotypic differentiation within *S. latissima*, which were documented between populations from Helgoland, Germany and Spitsbergen, Norway [8, 9]; and between populations of the core and southern limit distribution in the western Atlantic along the coastline of the USA [18]. Furthermore, recent studies indicate high genetic differentiation between Spitsbergen and Brittany, France populations and absence of admixture based on microsatellite genotypic data [6, 19]. In this study, we investigate how contrasting environments of origin (Roscoff, France, temperate Atlantic and Spitsbergen, Norway, Arctic) may be correlated with transcriptomic responses in *S. latissima*. Despite inhabiting a typical marine coastal environment in Roscoff, algal exposure to low salinities may occur occasionally at spring low tides in association with rain or runoff from land. In contrast, in Spitsbergen, in the Arctic, exposure to low salinities may be more frequent due to freshwater runoff from ice and glacier melting in the fjords [20]. In a changing climate, increased ice-melting and precipitation events may even amplify frequency and duration of exposure to hyposalinity conditions in the North Atlantic [21]. Moreover, these two locations are characterized currently by very different temperature conditions over the course of the year – *S. latissima* specimens living in Spitsbergen experience temperatures between approx. 0 °C to 8 °C [22] while specimens in Roscoff live in a warmer environment with mean seawater temperatures ranging between 9 °C and to 15 °C [23].
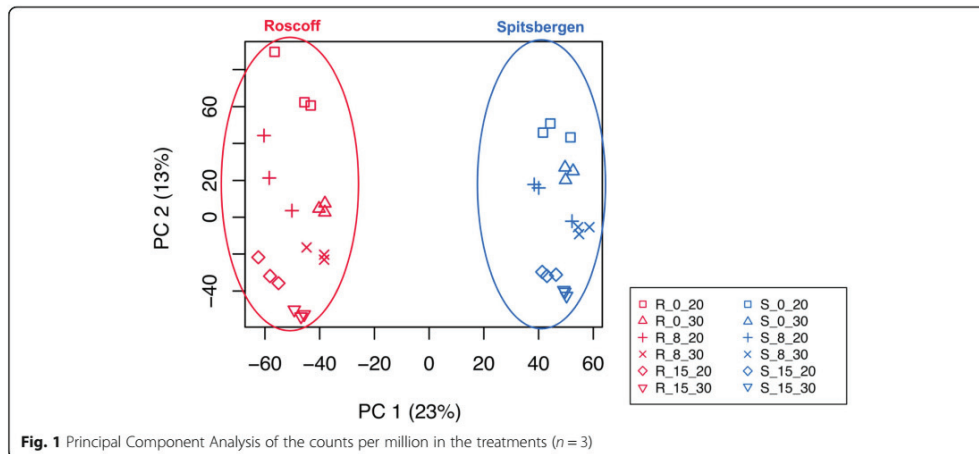
We hypothesized that sporophytic material isolated from these contrasting locations will show considerable differences in gene expression when exposed to the same abiotic stress conditions. We expected that material from Spitsbergen is better adapted to a combination of low temperature and low salinities while Roscoff material would perform better under high temperature and full marine salinity. Moreover we hypothesized that interactions between temperature and salinity might even enhance the differences between the response patterns of sporophytes from both locations.

Results

Read mapping, transcriptome quality assessment and Principal Component Analysis.

The number of reads per library ranged from 28 to 38 million with an average of 33 million reads. From the three transcriptome assemblies produced, the transcriptome assembly based on Roscoff samples was selected given that it presented the best overall remapping rates (~ 85%) compared to the cDNA library assembly based on Spitsbergen samples (~ 83%) and hybrid assembly created from both libraries (~ 83%). More precisely 85.2% of reads of the Roscoff cDNA library and 85.8% of reads of the Spitsbergen cDNA library remapped to the "Roscoff" de novo transcriptome. Additional mapping tests against the closest available genome of *Saccharina japonica* [24] led to a much lower remapping rate (39%) than on the de novo assembly. The raw transcriptome assembly consisted of 205,363 transcripts (or 135,959 Trinity genes) with an average contig size of 760 bp. Despite the apparent verbosity of the transcriptome, 90 % of total expression was present in 28,001 of transcripts (14% of the full transcriptome). The results of the contamination search revealed a very low percentage of potential contamination in our transcriptome (bacteria – 0.4%, oomycetes – 0.4%); therefore we did not remove any sequences. A majority of transcripts showed similarity to Phaeophyceae (87%). The results of the BUSCO analysis revealed a near-complete gene sequence information for our transcriptome with 262 complete BUSCO matches (86.5%), 23 fragmented BUSCOs matches (7.6%) and 18 missing BUSCOs (5.9%). Additional information concerning transcriptome statistics and annotation results is available in Additional file 1.

Our results from Principal Component Analysis (PCA) of counts per million showed that the replicates of the treatments clustered well (Fig. 1). The first axis (PC1)

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Fig. 1 Principal Component Analysis of the counts per million in the treatments ($n = 3$)

explained 23% of the variability and clearly differentiated between sporophytes originating in Roscoff and Spitsbergen. The second axis (PC2) explained 13% of the variability and differentiated between salinity x temperature treatments. Spitsbergen samples were separated by temperature (0 °C > 8 °C > 15 °C). Roscoff specimens from 0 °C and 8 °C first grouped by low salinity, then by control salinity and finally all 15 °C samples (both low and control salinity) grouped together.

Differential expression of Spitsbergen vs. Roscoff sporophytes at control conditions (8_30)

To investigate differences between the responses of Arctic versus temperate sporophytes suggested by the PCA (Fig. 1), we first looked at differences in gene expression between samples of Spitsbergen and Roscoff under control conditions (8_30): 958 differentially expressed genes (DEGs) (120 annotated with the UniProt Swiss-Prot database) were down-regulated in Roscoff in comparison to Spitsbergen material while 1766 (162 annotated) were up-regulated (Additional file 2).

We identified DEGs involved in nucleic acid metabolism, transport, cell wall synthesis and reorganization, general stress response, signaling and amino acid metabolism. In addition, nine genes involved in lipid metabolism were up-regulated in Roscoff control samples in contrast to only 2 genes in the Spitsbergen specimens (Additional file 2).

Several genes with cell wall-associated functions were differentially expressed between the control samples from the two locations. Namely, genes reported in higher plants such as callose synthases were up-regulated in Spitsbergen samples and UDP-D-xylose: L-fucose alpha-1,3-D-xylosyltransferase was up-regulated

in Roscoff samples with a $\log_2$-fold change of 6.3 among others. Moreover, genes with known cell wall-associated functions of brown seaweeds were found. Four transcripts of vanadium-dependent bromoperoxidase were up-regulated in Roscoff and two were up-regulated in Spitsbergen samples. In addition, a mannuronate C5-epimerase was up-regulated in Roscoff and two in the Spitsbergen control samples (Additional file 2).

Differential expression – salinity/temperature treatments compared to the control of algae from each geographical origin

Compared with the control (8_30) 4610 unique DEGs were found in specimens from Roscoff and 2966 from Spitsbergen. In the algae originating in Roscoff, the R_0_20 treatment caused the highest number of DEGs (47% - 3003), followed by R_8_20 (23% - 1491), R_15_20 (18% - 1160), R_15_30 (9% - 549) and finally R_0_30 (3% - 203) (Fig. 2 and Additional file 3). The low temperature/low salinity treatment accounted for almost half of the differential gene expression in the Roscoff samples.

In the sporophytes from Spitsbergen the pattern was different: the S_8_20 treatment induced the largest differential expression 34% (1374), followed closely by S_0_20 (30%- 1193), then by S_15_30 (20% - 801), S_15_20 (10% - 384) and finally by S_0_30 (7% - 274) (Fig. 2 and Additional file 3). Temperature treatments alone accounted for 12% of DEGs in sporophytes from Roscoff and for 27% in sporophytes from Spitsbergen (Additional file 3).

The Venn diagrams show that for algae from each location only few genes overlapped between treatments (Fig. 3). Moreover, a reduced percentage of DEGs per treatment was shared among sporophytes from the two sites. It varied from 4% for up-regulated

Fig. 2 Differentially expressed genes in response to temperature and salinity by geographical origin. On the right axis, total number of significantly up- and down-regulated DEGs compared to the control (8_30) of sporophytes from each location ($p < 0.001$; $log_2FC > 2$) is presented in grey bars. On the left axis and in colored bars number of DEGs compared to the control (8_30) according to functional categories of interest. Upper part of the graph displays up-regulated DEGs; the lower part down-regulated DEGs

genes in 15_20 to 17% for down-regulated genes in 8_20 (Additional file 3).

GO enrichment analysis

Several GO terms were enriched under the applied treatment conditions (Additional file 5): the numbers ranged from 44 (up-regulated DEGs in S_15_30) to 404 (down-regulated DEGs in S_8_20). Among up-regulated DEGs, the number of enriched GO terms was enhanced in R_8_20, R_15_20 and in R_15_30 compared to Spitsbergen samples, while at 0 °C more enriched GO terms were found in Spitsbergen (Additional file 5). The treatments resulted in a shift in several metabolic pathways such as lipid metabolism and nucleic acid metabolism, protein

Fig. 3 Venn diagrams of differentially up-regulated (↑) and down-regulated (↓) genes in the low salinity and in the temperature exposure treatments compared to the control (8_30) ($p < 0.001$; $log_2FC > 2$)
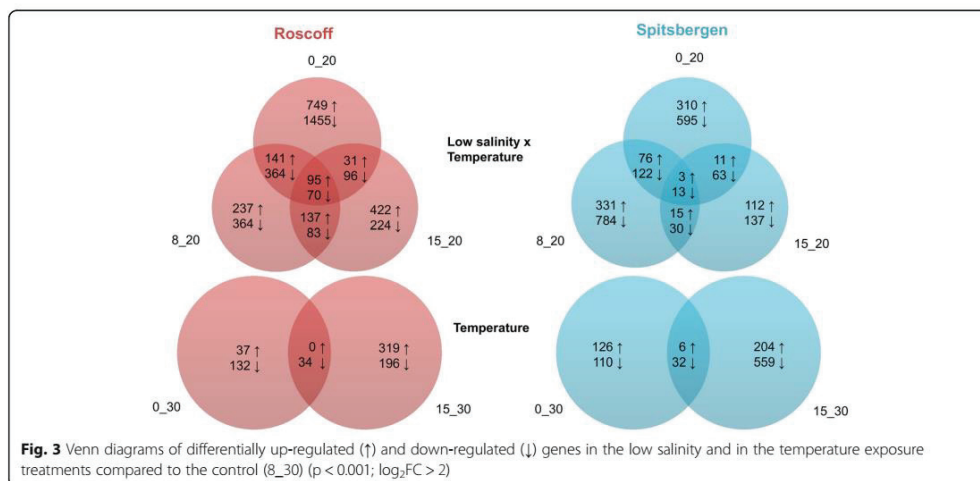
synthesis and modification, and in cell structure (Additional file 6). For the same treatment, some categories of enriched GO terms were only present in one of the location' samples, e.g. aminoacid metabolism among down-regulated DEGs at S_0_20, apoptosis among up-regulated DEGs at S_15_30. Other categories showed GO terms in higher quantity in specimens of one geographical origin compared to the other, e.g. more GO terms within lipid metabolism in the Roscoff samples among up-regulated DEGs at 0_20; more GO terms within metabolism in the Spitsbergen specimens among down-regulated DEGs at 15_20 (Additional file 6). Among enriched GO terms in the up-regulated DEGs at 0_20 and 15_20, there were some prominent differences between sporophytes from the two geographical origins in the categories cell signaling/communication and carrier proteins/membrane transport. At 0_20 more enriched GO terms were found in the category cell signaling/communication in Roscoff than in Spitsbergen specimens while more enriched GO terms functioning within carrier proteins/membrane transport were found in Spitsbergen samples; at 15_20 the pattern was reversed (Additional file 6).

Manual classification of DEGs

To identify the most important mechanisms involved in temperature and salinity acclimation and to investigate differences driven by geographical variation, we manually classified and examined all the annotated DEGs using the UniProt Swiss-Prot database as a starting point. The most obvious distinction was that in sporophytes from Roscoff more DEGs were regulated in several categories than the Spitsbergen isolates in response to the applied treatments.

Genes involved in transcription and/or translation were down-regulated at low salinity and decreased from R_0_20 (29 DEGs) to R_8_20 (19) to R_15_20 (2) but they were not differentially expressed in the temperature x control salinity treatments. In comparison, algae from Spitsbergen showed similar response at S_8_20 but less DEGs were regulated at S_0_20 (Fig. 2 and Additional file 7).

A large proportion of transcriptional regulation in the low salinity treatments was related to transport/ cell wall synthesis and reorganization (Fig. 2 and Additional file 7). In Roscoff, the highest number of DEGs was observed at R_0_20 (45), where the majority of genes were down-regulated (29) but 16 were up-regulated. While in Spitsbergen, the highest number of DEGs was promoted by S_8_20 (34) followed by S_0_20 (22); expression at 15 °C was similar in both salinity treatments (Fig. 2 and Additional file 7). A higher number of DEGs were identified within the class 'signaling' in Roscoff than in Spitsbergen samples, especially at 0_20 (Fig. 2 and Additional file 7).

Photosynthesis and pigment synthesis

In response to the hyposalinity treatments, a considerable number of DEGs were related to photosynthesis and photosynthetic components (93) in the algae from Roscoff, among these most were down-regulated (86). In comparison, in the control salinity treatments very few genes were differentially expressed in these categories (Fig. 2 and Additional file 7). However, in the samples from Spitsbergen, 43 DEGs were repressed at S_8_20, only 9 were expressed at S_0_20 and none at S_15_20 for the same categories. Similarly to Roscoff samples, very little transcriptional regulation was promoted by control salinity treatments (Fig. 2 and Additional file 7).

When comparing DEGs encoding transcripts related to photosynthesis between geographical origins, there were no noticeable differences in the magnitude of fold changes (Additional file 4). They were mostly down-regulated with a $\log_2$-fold change of around 2 and often triggered by the same treatments (Additional file 4). The most obvious difference was that several DEGs regulated in Roscoff were not observed in Spitsbergen individuals (Additional file 4). Namely, genes related to the light harvesting complex (e.g. chloroplastic chlorophyll a-b binding protein CP29.2, chloroplastic chlorophyll a-b binding protein 2) and electron transport (e.g. chloroplastic photosystem II 12 kDa extrinsic protein and chloroplastic oxygen-evolving enhancer protein 1) (Additional file 4).

Examples of genes with dynamic changes include magnesium chelatase subunit H, chloroplastic thioredoxin reductase NTRC (NTRC) and chloroplastic glutamate-1-semialdehyde 2,1-aminomutase (Additional file 4). Magnesium chelatase was down-regulated in all low salinity treatments in Roscoff samples but it was not regulated in Spitsbergen specimens (Additional file 4). Moreover, NTRC was down-regulated in R_0_20 samples and glutamate-1-semialdehyde 2,1-aminomutase was down-regulated in R_8_20, R_15_20 and in S_8_20 (Additional file 4). Four genes encoding chloroplastic pheophorbide *a* oxygenases were differentially expressed in our study. Two genes were induced in the R_0_20 and R_8_20 treatments; one of them was also induced in R_15_20. However, a third gene was repressed in R_0_20 and a fourth one in the S_8_20 sporophytes (Additional file 4).

Concerning the violaxanthin cycle, chloroplastic violaxanthin de-epoxidase was down-regulated in the S_8_20, R_0_20 and R_8_20 treatments while chloroplastic zeaxanthin-epoxidase was repressed in S_8_20 and R_0_20 specimens (Additional file 4).

Regarding the results of pigment analysis (additional file 8), violaxanthin had a significantly higher concentration in R_0_20 than in R_0_30 ($p = 0.010$, pairwise comparisons, Bonferroni adjustment), but no differences were found for 8 °C. In Spitsbergen samples,

violaxanthin contents were significantly higher in the low salinity treatments than in the control ($p = 0.025$). The zeaxanthin content did not significantly change in Roscoff samples, however in specimens from Spitsbergen, zeaxanthin concentration at 0 °C was significantly higher than at 15 °C ($p = 0.031$, pairwise comparisons, Bonferroni adjustment).

Carbon metabolism

Concomitantly with repression of genes important for photosynthesis, several enzymes related to carbon metabolism were down-regulated under hyposalinity. Namely, chloroplastic phosphoribulokinase and chloroplastic fructose-1,6-bisphosphatase were down-regulated in R_8_20 samples while transketolase, cytosolic fructose-bisphosphate aldolase and cytosolic glucose 6-phosphate isomerase were repressed under the same conditions in Spitsbergen samples. Furthermore, chloroplastic ribulose-1,5 bisphosphate carboxylase/oxygenase large subunit N-methyltransferase was repressed at 8_20 in Spitsbergen and Roscoff specimens (Additional file 4). Moreover, cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was down-regulated at R_15_20 but up-regulated at 0 °C and 15_30 in Spitsbergen specimens (Additional file 4).

Stress responses

In Roscoff samples, a higher number of DEGs involved in oxidative stress response stress were expressed in the low salinity compared to the control salinity treatments. However, there was a mixed response with both induction and repression of gene expression (Fig. 2 and Additional file 7). Some reactive oxygen species (ROS) related genes were down-regulated, e.g. chloroplastic thioredoxin reductase NTRC (NTRC) in R_0_20 and heme-binding peroxidase at all low salinity treatments (Additional file 4). The response of the latter might be explained by the fact that it was already constitutively expressed in the control 8_30. Heme-binding peroxidase expression level in the control samples (Roscoff - 98.7 transcripts per kilobase million (TPM); Spitsbergen - 217 TPM) was higher than the average of all expressed genes (Roscoff - 21.6 TPM; Spitsbergen - 21.6 TPM) (Additional file 9). Nearly no ROS scavenging related genes were up-regulated in R_0_30 sporophytes, whereas Spitsbergen material showed some induction (Fig. 2 and Additional file 7). On the other hand, more ROS related DEGs were induced at 0_20 and less at 15 °C in Roscoff samples compared to Spitsbergen material (Fig. 2 and Additional file 7).

Superoxide dismutase (Fe) was down-regulated in S_8_20, R_8_20 and R_15_20 specimens, a cytosolic L-ascorbate peroxidase (APX) was down-regulated in S_8_20, R_0_20 samples, furthermore a chloroplastic APX

was up-regulated in R_8_20 (Additional file 4). Two alternative oxidases were differentially expressed in our setup. The chloroplastic ubiquinol oxidase was induced at 0 °C in Spitsbergen samples and also at R_0_20 while it was repressed at 15 °C in Roscoff specimens and the alternative oxidase (mitochondrial) was up-regulated at 0_20 in both Roscoff and Spitsbergen samples (Additional file 4). A peptide methionine sulfoxide reductase (MsrA) gene was up-regulated in response to all low salinity treatments in Roscoff samples and in S_0_20 treatments, however another gene was down-regulated in S_8_20 (Additional file 4). The chloroplastic lactoylglutathione lyase was repressed in R_0_20, R_8_20 and S_8_20 samples (Additional file 4). Glutathione S-transferases (GSTs) were induced in R_0_20 and R_8_20 and repressed in 15 °C (around − 2 log2FC) samples (Additional file 4). In Spitsbergen samples, GSTs showed the same pattern with the exception that in the 0_30 treatment they were up-regulated in Spitsbergen and showed no changes in sporophytes from Roscoff (Additional file 4). Twenty genes encoding vanadium-dependent bromoperoxidases were differentially expressed in our experiment. They were mostly down-regulated in R_0_20 and R_8_20 and up-regulated at 15 °C in Roscoff samples. However, in the Spitsbergen samples, they were induced at S_8_20 and repressed in the 15 °C treatments. Moreover, we did not find any vanadium-dependent iodoperoxidases (Additional file 4). When looking at the TPM, we identified nine antioxidant genes constitutively expressed in the control from both locations and three were expressed at highly different levels between Roscoff and Spitsbergen samples − two (chloroplastic thioredoxin-like protein HCF164 and glutaredoxin-C2) presented higher expression in Roscoff than in the Spitsbergen samples while the reverse was observed in another one (heme-binding peroxidase) (Additional file 9). Concerning chaperones, HSPB1-associated protein 1 was repressed in R_0_20 while 10 kDa chaperonin was repressed in R_8_20 samples. Chaperone protein ClpB 2 was repressed in S_8_20 and the 78 kDa glucose-regulated protein was repressed in S_15_30 samples (Additional file 4). Furthermore, we identified sixteen chaperones constitutively expressed in the control of Roscoff and Spitsbergen samples and three chaperones with clearly different expression profiles between Roscoff and Spitsbergen samples - two at higher expression levels in the Spitsbergen samples (heat shock 7 kDa protein 5 and heat shock protein 9–1), one was expressed at higher levels in the Roscoff ones (assembly chaperone of rpl4) (Additional file 9).

Salinity specific response

Mannitol 2-dehydrogenase was among the expressed genes but it was not significantly differentially expressed.

No other genes related to mannitol biosynthesis were found. The lack of differential expression may be the result of constitutive expression in the control specimens (Additional file 9). Proline dehydrogenase 1 (mitochondrial) was also among the expressed genes but it was not differentially expressed. Moreover, its expression level, in TPM, was below the average.

Glycerophospholipid metabolism

We detected three desaturases down-regulated in low salinity treatments (Additional file 4). Stearoyl-CoA desaturase 5 was down-regulated at 0_20 in Roscoff and Spitsbergen samples, sn-2 acyl-lipid omega-3 desaturase (FAD7) was down-regulated at S_8_20, R_0_20 and R_8_20 and a delta-fatty-acid desaturase (FAD2) was down-regulated at R_0_20 samples. A choline kinase was down-regulated in S_8_20, R_0_20 and R_8_20 sporophytes. One gene encoding oxygen-dependent choline dehydrogenase was down-regulated at S_0_20 and S_8_20. Another gene was repressed at 0 °C and S_8_20 in Spitsbergen, R_0_20 and R_8_20 samples (Additional file 4).

Discussion

Out of the 6114 DEGs in our study (unique DEGs versus control in specimens from both locations), only 756 (12.4%) could be functionally annotated using the UniProt Swiss-Prot database. Therefore, almost 90% of the genes regulated in *S. latissima* under a combination of temperature and salinity stress remain unknown. These results highlight the potential and need to discover new metabolic pathways required by the species to tackle environmental changes and further support brown algae as a relevant piece in understanding evolutionary history [25].

The number of DEGs in our study is comparable to the numbers reported in other studies, i.e. in *Desmarestia anceps* in response to light, temperature and CO_2 [between 337 and 3255 DEGs in the comparison to the control [26];].

Transcriptomic differences driven by geographical variation

The response of sporophytes from Arctic Spitsbergen and temperate Roscoff differed in many aspects. These differences became apparent during all applied analyses (PCA, differential expression and GO enrichment). A higher number of DEGs was found in Roscoff specimens than in the Spitsbergen ones when comparing treatments against the respective control. In addition, there was little overlap between DEGs under the same exposure conditions in the sporophytes originating in distinct geographical sites. These results and the extensive disparity among GO enrichments demonstrate that not only the magnitude of the response differs between specimens of the two locations but also that the metabolic reorganization was performed differently. Moreover, several genes were differentially expressed between the control samples of Spitsbergen and Roscoff, even though they were cultured under the same conditions.

Physical properties of the cell might be affected under changing ambient conditions [27, 28]. DEGs with cell wall and cytoskeleton-associated functions, which are critical in the cell's adjustment to temperature and osmotic fluctuations, were observed. An example of a DEG between the control samples is callose synthase that catalyzes the synthesis of callose in plants [29]. Callose regulates permeability in plasmodesmata, which play a role in cell to cell communication and transport of macromolecules. Callose accumulation is affected both by developmental cues and stress in plants [30]. Recently, an immunolocalization study found a callose (β-(1,3)-glucan) epitope in the cell walls of the brown alga *Fucus vesiculosus* and in the sieve plates of *Laminaria digitata* [31]. Furthermore, cell wall adjustment was identified as one of the main processes in acclimation to freshwater in an *Ectocarpus siliculosus* sister species [32]. The same study also connected changes in transcripts such as mannuronate C5-epimerase and vanadium dependent bromoperoxidases (V-BPOs) with differences in morphology between the sister species [32]. This is comparable to our study in *S. latissima*, as sporophytes used in our experiment had different phenotypes since an early stage of cultivation (Additional file 10). Sporophytes originating in Spitsbergen were more elongated and narrower than sporophytes from Roscoff and attained a lower fresh weight after three months of cultivation. In the comparison of exposure treatments to the respective controls, V-BPOs were mostly down-regulated at 0 °C and 8 °C low salinity and up-regulated at 15 °C in the Roscoff samples. However, in algae from Spitsbergen, they showed a contrasting pattern – induction at 8 °C low salinity and repression at 15 °C. Contrary to our data, V-BPOs did not change in response to hyposalinity stress in *S. japonica* [33] nor in *Ectocarpus siliculosus* [34], however they are involved in the response to other environmental stressors in kelps, e.g. in *Macrocystis pyrifera* [35] and in *Laminaria digitata* [36]. Furthermore, differential gene expression of V-BPOs has been previously identified in *S. latissima*: the interaction of temperature, light and UV-radiation resulted in a mixed response after two weeks exposure, however, V-BPOs were mostly up-regulated at higher temperatures [16] and down-regulated in response to low temperatures after 24 h [15]. Regulation of V-BPOs in our study might be connected to cell wall adjustments in response to osmotic stress as V-BPOs have been reported to promote the binding of phenolic substances to alginates in algal cell walls [37].

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Moreover, we found several genes associated with lipid metabolism, mostly induced in Roscoff control samples. Changes in lipids within membranes will affect its composition, integrity and fluidity [38]. Altogether, our results indicate that cell wall and membrane adjustment might be important mechanisms in the acclimation of *S. latissima* to contrasting environmental conditions.

Differences in treatment responses driven by geographical origin included the expression of transcripts encoding photosynthesis related components. Although photosynthesis was repressed under low salinity in samples from both locations, more DEGs were down-regulated in specimens from Roscoff than from Spitsbergen. Additionally, a key enzyme in chlorophyll catabolism, pheophorbide *a* oxygenase [39], was induced in Roscoff samples but repressed in Spitsbergen material. Also, magnesium chelatase and NTRC, which play key roles in the regulation of chlorophyll metabolism [40] were only differentially expressed in Roscoff samples. This suggests that hyposalinity stress was more severe for sporophytes originating in Roscoff than in Spitsbergen, demanding a stronger reduction in photosynthesis. This might be explained by the fact that in Roscoff algae are exposed to a stable saline environment and are less able to adjust to salinity changes. Moreover, at lower temperatures photo-inhibition of photosynthesis may occur [41], a process that might be more frequent in algae seldom exposed to cold temperatures, such as in specimens from Roscoff.

Furthermore, both GO enrichment and DEGs results showed enhanced cell signaling at 0_20 in Roscoff samples compared to Spitsbergen and at 15 °C in Spitsbergen compared to Roscoff material. This pattern suggests that the low temperature and low salinity conditions tested are not commonly encountered in Roscoff and therefore required a stronger signaling response than in Spitsbergen samples in order to trigger physiological adjustments. Differential expression of signaling related genes as a result of local adaptation has so far, to our knowledge, not been described in algae. However, in the marine realm, it has been observed between closely related species of mussels with distinct heat tolerance [42] and between populations of a species of killifish in response to local salinity variability [43].

Responses to salinity and temperature changes
Several metabolic pathways were involved in the response to temperature and salinity variation in *S. latissima*, demonstrating the relevance of these factors to its physiology and ecology. To capture the responses to relevant ecological variation at the sites, the levels of the applied stress corresponded to levels experienced at these sites (Roscoff [23], Spitsbergen [22]) and fall well within the physiological limits of the species [10–13].

Thus, the applied stress was only mild, which might explain why some classic stress responses were not observed, such as strong up-regulation of heat shock proteins, antioxidant enzymes and catabolic processes, as previously observed in *S. latissima* in response to high light and high temperature [16] and in several other macroalgae in response to severe stress levels [44–46]. Still, our results help to unravel how acclimation to temperature and salinity changes is achieved in *S. latissima*. Pathways involved included signaling, photosynthesis and pigment synthesis, stress related enzymes, transport and cell wall synthesis and reorganization.

The analysis of DEGs shows that the low salinity treatments had a higher impact on the number of genes regulated than temperature alone in sporophytes from both locations; however, the proportion and the magnitude of impact were different between treatments. Given that the algae had already been exposed for eight days to the experimental temperatures before the onset of the salinity treatment, the limited response to temperature may indicate that the sporophytes were already acclimated [47] and observed differences are only the long-term changes in gene expression and not short-term stress responses. In turn, salinity driven responses were only evaluated after 24 h and therefore the differential impact of temperature and salinity on gene expression profiles might be driven by temporal variation in responses. As we assessed only one sampling point, we cannot infer about the temporal dynamics of the response and further studies are necessary to understand the regulatory kinetics in the acclimation to these abiotic factors.

Only a few DEGs overlapped between treatments applied to algae from the two locations. This indicates that a common response to salinity or temperature stress was not evident, but interactions between the two factors were apparent. The higher temperature (15 °C) triggered a smaller response than 0 °C, especially in the Roscoff sporophytes.

We observed down-regulation of photosynthesis and carbon assimilation which seems to be a common response pattern to salinity changes as it was also found in a diatom, another brown alga and terrestrial plants [34, 48, 49]. Additionally, we observed down-regulation of enzymes contributing to the xanthophyll cycle at S_8_20 and R_0_20. However, we were not able to link gene expression with the pigment content measured in our samples (Additional file 8). The xanthophyll cycle is an important photoprotection mechanism in vascular plants, green and brown seaweeds, and related to the de-epoxidation of violaxanthin to zeaxanthin, leading to dissipation of excess excitation energy [50]. In our study, down-regulation of xanthophyll cycle enzymes might have been connected to a reduced photosynthetic capacity thus reducing the need for photoprotection. The

dark operation of the xanthophyll cycle has been observed in response to abiotic stressors in another brown alga, *Pelvetia canaliculata* [51]. However, to the best of our knowledge, our study is the first potentially implicating the xanthophyll cycle in salinity stress responses.

Heat shock proteins (HSPs) are often induced under abiotic stress [52]. Previously, 13 genes encoding HSPs were reported to be differentially expressed in *S. latissima* under temperature and light stress after 24 h [15]. Unexpectedly, we only found four HSPs DEGs regulated after eight days of acclimation to temperature (0 °C and 15 °C). This indicates that HSPs are rather induced in short-term responses but reduced in longer term acclimation, which was also shown by Heinrich et al. [16]. Alternatively, it suggests that HSPs are not intensively involved in response to cold, as already reviewed in Sørensen and Loeschcke [53], and that the highest temperature tested, 15 °C, was not high enough to induce HSPs. This hypothesis is supported by reports on a stronger transcriptional regulation of HSPs in response to high (17 °C) than to low temperature (2 °C) [15]. Moreover, Dittami and colleagues found three HSPs, one down-regulated and two up-regulated in a hyposalinity treatment in *E. siliculosus* [34]. Taken together, these results suggest that HSP regulation in response to salinity variation takes place to a lesser extent than in response to temperature.

Genes involved in reactive oxygen metabolism often play a key role in stress responses [54, 55]. The main ROS scavenging enzymes in plants are superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) [55]. However, those did not seem to be involved in the response to stress in our study as they were either down-regulated (SOD, APX) or not regulated (CAT). Lactoylglutathione lyase, that catalyzes the synthesis of methylglyoxal, was also repressed under hyposalinity (R_0_20, R_8_20 and S_8_20). The reduction in methylglyoxal production might be a stress tolerance strategy as was already reported in plants [56] and in the kelp *Lessonia spicata* in response to desiccation [57].

On the other hand, some stress related genes were induced in response to the temperature and salinity levels tested in our experiment. Alternative oxidases can reduce ROS production by diverting electrons from the electron transport chains to produce water from oxygen [55]. Two alternative oxidases were mostly up-regulated in our study. One gene encoding an ubiquinol oxidase was up-regulated at 0 °C in Spitsbergen specimens, up-regulated in Roscoff sporophytes at 0_20, but down-regulated at 15 °C. A second gene was also up-regulated at 0 °C in Spitsbergen samples. Furthermore, peptide methionine sulfoxide reductase has an important function as a repair enzyme for proteins that have been inactivated by oxidation [58]. It was also shown to be up-

regulated in *Laminaria digitata* in response to biotic stress [59]. The low salinity treatments provoked up-regulation of MsrA - at 0_20 in Spitsbergen samples, and at all low salinity treatments in Roscoff samples, indicating that Spitsbergen specimens were less affected by low salinity.

Glutathione S-transferases are enzymes that conjugate glutathione with electrophilic components, playing several roles in eukaryotic cells such as detoxification of xenobiotics, metabolism of aromatic amino acids and lipids [60]. In algae, they have been measured in response to environmental pollution and to abiotic stress [36, 61]. In our data, GSTs were up-regulated at 0 °C and 8 °C and down-regulated at 15 °C in samples from both locations. Although GSTs play different roles in cell metabolism and therefore more studies are necessary to determine their exact function, they seem to be linked to temperature acclimation in the conditions of our experiment.

In our study, we failed to detect changes in transcripts coding for enzymes involved in the synthesis of the known osmolyte in brown algae, mannitol [62] and proline, with a similar function in plants and diatoms [63, 64]. This finding is contrary to previous results on the down-regulation of a mannitol 1-phosphate dehydrogenase and the induction of proline dehydrogenase in response to a hyposalinity treatment in *E. siliculosus* [34]. However, oxygen-dependent choline dehydrogenase was down-regulated at 0_20 and 8_20 in Roscoff and Spitsbergen' sporophytes. This enzyme is involved in the biosynthesis of glycine betaine, an osmoprotectant in terrestrial plants [65]. However, to the best of our knowledge, there are no reports of similar functions in Phaeophyceae, even though the compound has been shown to be present in brown algae [66]. If this osmolyte would have a similar function in *S. latissima*, we would expect its biosynthesis to be induced. All together, we can speculate that *S. latissima* does not rely on synthesis of the osmolytes proline and mannitol during short-term responses to salinity. Metabolomics and proteomics studies would help us understand if this regulation is not part of the response or if it is post-translational.

Phospholipids are an important component of cell walls, and modifications of their composition leads to adjustment of membrane fluidity in response to environmental change [38, 67]. Choline kinases catalyze the first step in phosphatidylcholine biosynthesis, a phospholipid present in eukaryotic cell membranes [68]. Choline kinases have been suggested as key regulatory enzymes in salt stress response in *Arabidopsis* [69] and they were partially repressed in response to hyposalinity in our study - S_8_20, R_0_20 and at R_8_20. Moreover, we found three desaturases regulated in our experiment - FAD7, FAD2 and a stearoyl-CoA desaturase. Desaturases

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are enzymes that catalyze the biosynthesis of polyunsaturated fatty acids [67]. Changes in their expression in *Ectocarpus* were associated with adaptation to freshwater [32]. Moreover, the expression of FAD7 in transgenic tobacco increased cold tolerance in the plant by increasing levels of trienoic fatty acids [70]. An increase in unsaturation of fatty acids in winter has also been reported in polar macrophytes [71]. However, the desaturases genes differentially expressed during our study were downregulated and restricted to the low salinity treatments (0_20 and 8_20).

The limited number of genes involved in known stress responses regulated in this study, namely HSP and proteins with antioxidant activity, are in accordance with responses of the brown alga *E. siliculosus* and the red alga *Chondrus crispus* to hyposalinity [34, 61]. A suggested explanation is that post-transcriptional regulation is a more important mechanism than transcriptional regulation modulating stress related proteins. This is supported by the identified mismatch between gene expression and protein data in *Arabidopsis* in response to salt stress [71]. Another reason might be that the strong down-regulation of photosynthesis, namely chlorophyll binding proteins, reduced the amount of energy reaching the photosynthetic reaction centers and therefore reduced the production of reactive oxygen species [72]. However, an alternative explanation is that some classic stress genes are already constitutively expressed in the control (Additional file 9) and given that we used a stringent threshold ($p < 0.001$, $\log_2 FC > 2$) for our differential expression analysis, we failed to detect smaller variations. This constitutive expression may allow *S. latissima* to acclimate to stress quickly and might be part of its broad tolerance to stress.

Ecological implications

The limited response to temperature changes in sporophytes irrespective of their origin suggests a high tolerance to temperature of *S. latissima*, which is mirrored in its broad latitudinal and vertical distribution [6, 7]. Although *S. latissima* is currently not exposed to seawater temperatures of 15 °C in the Arctic, this temperature did neither promote extensive transcriptomic changes nor stress responses. However, in comparison to the Roscoff specimens, it showed a larger overall response and indeed a higher number of antioxidant genes were regulated in Spitsbergen material at 15 °C.

Hyposalinity had a higher impact at the transcriptional level than temperature alone. This suggests that acclimation to this stressor might be more costly and therefore has a higher potential to impact growth at these locations. The interaction between low salinity and low temperature was especially stressful for Roscoff sporophytes and triggered an extensive repression of photosynthesis, with presumably great impact on physiology and growth of the specimens. On the other hand, higher temperatures appear to ameliorate the hyposalinity stress. This is the first study investigating transcriptomic responses to salinity in *S. latissima* and even in physiological studies this abiotic factor has been remarkably underrepresented. Given our results, to better understand current local physiological performance and its modulation by future global change, more research targeting salinity and its interactions with other factors is necessary.

Conclusions

Resilience of macroalgae stands to climate change was identified as population-specific in some studies e.g .[73]. The use of the transcriptomic data to unravel local adaptation and/or phenotypic plasticity is a recent but promising strategy [42, 74, 75]. In brown algae, Jueterbock et al. [76] demonstrated that the expression of heat shock proteins was population-specific in *Fucus serratus*. Similarly Mota et al. [77] described differences between cold and warm edge populations of *Fucus vesiculosus* in photosynthetic efficiency and expression of heat shock proteins, and Ritter et al. [78] showed that two populations of *E. siliculosus* presented different responses to copper stress. However, in these studies real-time PCR was used, a technique that restricts the analysis to a few genes of interest. RNA-sequencing allows uncovering large-scale transcriptomic responses without being limited to known sequences, and thus is a very powerful strategy especially in non-model organisms with limited genomic information. However, given the relatively high costs of this technology, experiments combining several factors and biological replicates are nearly unattainable. A previous study in *S. latissima* from Spitsbergen revealed that field and culture material responses to UV-radiation under different temperatures involved similar processes, even though in different intensities [17]. Therefore studies such as ours may provide the basis to select the abiotic factors and populations most likely contributing to the understanding of acclimation mechanisms in brown algae and giving insights into the modulation of responses along geographical gradients. We provide a large-scale transcriptomic analysis of temperature and salinity acclimation mechanisms in sporophytes originating in the central and northern distribution of the sugar kelp, *Saccharina latissima*. The differences between transcriptional responses of cultured sporophytes from the two locations to stress fit to a certain extent with our initial hypothesis that Roscoff sporophytes would perform better at higher temperatures and be affected more intensively by hyposalinity and that in turn Spitsbergen sporophytes would perform better at lower temperatures and at low salinities. The high number of DEGs reported between the

respective controls together with their distinctive morphology even after cultivation under the same conditions further support that responses in these two geographical origins have diverged and that they might have adapted to their local conditions. The temperature tolerance of the Spitsbergen uniparental sporophytes observed here might confer resilience to a warming Arctic. Moreover, its better performance under hyposalinity conditions might be an advantage over the Roscoff sporophytes in case of a poleward shift of the latter. To better understand population transcriptomics in *S. latissima* future studies should include experiments with specimens originating in other sites in other populations, ideally from both field and cultivated material, and more genetic variability from the same locality is needed. Especially, information about populations living closer to their physiological limits would further elucidate the mechanisms underlying the broad tolerance and therefore broad distribution of the species.

Methods

Algal material

Young sporophytes of *S. latissima* were raised from stock cultures of uniparental male and female gametophytes at the Alfred-Wegener-Institute Helmholtz Centre for Polar and Marine Research (AWI, Bremerhaven, Germany; stock culture 3425 and 3426 Roscoff; 3123 and 3124 Spitsbergen) according to the protocol of Heinrich et al. [15]. The parental sporophyte material from which unialgal clonal gametophyte cultures were isolated [79] was collected in the wild and was identified by Andreas Wagner (Roscoff, Brittany, France; 48° 43′ 39″ N, 3° 59′ 13.2″ W) and by Christian Wiencke (Spitsbergen, Norway; 79°N, 11°E). *Saccharina latissima* is a kelp species distributed along the European coastline and easily and unmistakably to identify. No voucher specimens of the sporophytes were created. As material from Spitsbergen and Roscoff was collected in 1991 and 2013, respectively, it does not fall under the Nagoya legislation which regulates the access and benefit sharing (ABS) of biological resources since 12 October 2014. Sporophytes of both locations were grown aerated in glass beakers at 8 °C under a photon fluence rate of 20 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR) (Mitras Lightbar Daylight 150, GHL, Germany) with a 18 h light: 6 h dark photoperiod. Algae were cultivated for three months in sterile seawater enriched with Provasoli [80] with an absolute salinity (S$_A$) [81] of ~ 30 until they reached an average fresh weight of 0.13 g (7–9 cm) for Spitsbergen sporophytes and 0.58 g (5–7 cm) for Roscoff sporophytes.

Experimental set-up

At the start of the experiment, sporophytes were kept at the respective experimental temperature (0 °C, 8 °C and 15 °C) in temperature controlled rooms. Temperatures were chosen to mirror local conditions at sampling sites (Spitsbergen: 0 °C to 8 °C [22], Roscoff: 9 °C to 15 °C [23]) and to allow for a balanced experimental design where low temperature and high temperature treatments vary equally (+/– 1 °C) from the control temperature (8 °C). Control conditions were also selected with respect to optimal growth requirements of *S. latissima* [10, 11]. After one week, per each temperature, sporophytes were divided into a low salinity treatment of S$_A$ 20 or kept under the control salinity (S$_A$ 30). S$_A$ 20 seawater was obtained by adding deionized water. We set the hyposalinity stress level at S$_A$ 20 to guarantee that it was within tolerance levels [12, 13] and to represent an occasionally ecological relevant level in Spitsbergen following snow and ice melt run-off [20].

Each treatment was applied to 5 replicate aerated beakers (5 L) with 12 sporophytes each. After 24 h, whole sporophytes for RNA extraction ($n = 3$) and pigments quantification ($n = 5$) were taken. Sporophytes were frozen in liquid nitrogen and stored at – 80 °C until further use. Remaining sporophytes were stored for further analysis not covered in this manuscript.

Pigment quantification and statistical analysis

Pigment analysis was performed according to the method described in Bollen et al. [82]. Then we calculated the percentage of initial values (before salinity acclimation) of violaxanthin and zeaxanthin concentrations. All data were tested for normality using the Shapiro-Wilk normality test and for homogeneity of variances using the Levene's test. Similarity of pigment contents were tested using a two-way ANOVA with the fixed factors temperature and salinity. Significant differences and interaction of means were compared with the post hoc Tukey test (HSD). When data failed to comply with normality and homogeneity of variances assumptions, we performed a non-parametric test (Mann-Whitney U or Kruskal-Wallis test). All statistical analyses were carried out using SPSS software version 24 (IBM, Armonk, USA). The significance level for all analyses was set at α = 0.05.

RNA-sequencing and data processing

Data obtained, quality control and trimming

Total RNA extraction was conducted using the method described in Heinrich et al. [83]. RNA quality was analysed by the NanoDrop ND-1000 UV-Vis Spectrophotometer and Agilent 2100 Bioanalyzer (Agilent Technologies, Germany). cDNA libraries were prepared with an Illumina TruSeq RNA Library Prep Kit according to the manufacturer protocol and sequenced in triplicates. The libraries were sequenced on an Illumina Hiseq 2500 and 75 bp paired reads were clipped using default values of the Illumina software.

Raw reads were quality controlled by FastQC v. 0.11.5 [84] and quality filtered using Trimmomatic v. 0.36 [85]. Quality filtering was performed using the following parameters: leading 3, trailing 3, sliding window 4:15, minlen 30.

Assembly

Reads from all treatments were assembled de novo all together and separately for sporophytes from Spitsbergen and Roscoff. The assemblies were performed with Trinity v 2.4.0 including the reads normalization step corresponding to the Trinity implementation of the diginorm method. The assembler was run with default parameters [86]. The quality of the transcriptome assemblies was evaluated by using BUSCO v2.0 [87] with eukaryote dataset (OrthoDB v9.1]. To check for potential contamination, bacterial and oomycete sequences obtained from Genbank were compared to the transcriptome assembly by sequence similarity search (blastn).

Mapping and DEG analysis

The reads from both Roscoff and Spitsbergen samples were pseudo-aligned with Salmon [88] against the three de novo assembled transcriptomes based on both libraries, Roscoff cDNA library only and Spitsbergen cDNA library only. A PCA plot of the counts-per-million, followed by a $\log_2$ transformation, of all treatments was generated by a Trinity script. Differential expression was calculated using DESeq2 [89] at Trinity's gene level with an adjusted level of $P \leq 0.001$ and a $\log_2$-fold change of at least 2 indicating significance. One differential expression analysis was performed comparing the control samples of Spitsbergen to the control samples of Roscoff. A second analysis compared each exposure treatment to the control of sporophytes from each location. The combination of three temperatures – 0 °C (0), 8 °C (8) and 15 °C (15) and two salinities – S_A 20 (20) and S_A 30 (30) resulted in five treatments compared to the control (8_30) per site of origin in a total of 10 comparisons (Spitsbergen (S): S_0_20, S_0_30, S_8_20, S_15_20, S_15_30; Roscoff (R): R_0_20, R_0_30, R_8_20, R_15_20, R_15_30).

Tools were executed using the scripts included in the Trinity package v 2.4.0 [86].

Functional annotation and enrichment

Functional annotation was performed using the Trinotate functional annotation pipeline [90] with the UniRef90 database as additional reference to the default database Uniprot Swiss-Prot database (all databases up to date in October 2017). To investigate the function of significantly up- and down-regulated genes, Gene Ontology (GO) enrichments were conducted using GOseq [91]. Enriched GO terms were summarized with CateGOrizer using the EGAD2GO classification file [92]. For exploring constitutively expressed transcripts within the control, normalized read counts, given as transcripts per kilobase million (TPM), were analysed, following the approach of Iñiguez et al. [26]. Venn diagrams were produced through a webtool [93].

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12870-019-2124-0.

Additional file 1. Summary of transcriptome statistics and of transcript annotations in the public databases.

Additional file 2 List of DEGs found in the comparison of control (8_30) between Spitsbergen and Roscoff samples; $P \leq 0.001$ and $\log_2$-fold change > 2.

Additional file 3. Number of DEGs for each pairwise comparison per location; $P \leq 0.001$ and $\log_2$-fold change > 2; Percentage of DEGs shared between Roscoff and Spitsbergen samples per treatment.

Additional file 4. List of DEGs found in the exposure treatments against control (8_30) of each location; $P \leq 0.001$ and $\log_2$-fold change > 2.

Additional file 5. Number of significantly enriched GO terms among DEGs. Comparison of exposure treatments against control (8_30) of each location.

Additional file 6. Functional categories derived from enriched GO terms of differentially expressed genes of the treatments compared to the control (8_30): A) 0 °C treatments, B) 8 °C treatments, C) 15 °C treatments; sporophytes from Spitsbergen are represented in the blue bars and specimens from Roscoff in the red bars. Classification after EGAD2GO using cateGOrizer.

Additional file 7. Manual classification of DEGs compared to the control (8_30) according to functional categories of interest. ↑ indicates up-regulated DEGs, ↓ down-regulated DEGs.

Additional file 8 Results of the two-way ANOVA and graphic representation for effects of temperature, salinity and their interaction on the pigments measured after 24 h of temperature and salinity exposure. Statistically significant values are indicated by asterisks ($P < 0.05$). When data failed to comply with normality and homogeneity of variances assumptions, results of a non-parametric test (Mann-Whitney U or Kruskal-Wallis test) are showed.

Additional file 9. Transcript per million (TPM) counts of the control treatment (8_30) of sporophytes from each geographical origin corresponding to genes encoding chaperones, antioxidant proteins and salinity stress expected genes. In bold TPM values of genes considered constitutively expressed in the control, in red genes with TPM values considered highly different between samples from the two locations.

Additional file 10. Phenotypic differences between sporophytes from Roscoff and Spitsbergen before the start of the experiment. A) Spitsbergen, B) Roscoff.

Authors' contributions

Funding

Availability of data and materials

The Illumina sequence reads generated during the current study have been deposited in the Array express repository [94], under the accession number E-MTAB-7348.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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5 Physiological responses to temperature and salinity variation in juvenile sporophytes of *Saccharina latissima* from Roscoff, France

Title: Physiological responses to temperature and salinity variation in juvenile sporophytes of the kelp *Saccharina latissima* from Roscoff, France

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Abstract

The kelp *Saccharina latissima* experiences a wide range of environmental variation along its geographical and vertical range. Temperature and salinity are two of the main abiotic factors influencing growth, photosynthesis and biochemical composition. Moreover, interactive effects might modify the results described for single effects. In shallow water coastal systems exposure to increased temperature and low salinity are expected as consequence of global warming and increased precipitation and coastal run-off.

To understand the acclimation mechanisms of *S. latissima* to changes in temperature and salinity, we performed a laboratory experiment in which juvenile sporophytes were exposed to a combination of temperature (0 °C, 8 °C and 15 °C) and salinity levels (SA 20 and SA 30). After a temperature acclimation of seven days, sporophytes were exposed to low salinity (SA 20) for a period of eleven days. Growth, and maximal quantum yield of photosystem II (F_v/F_m), pigment, mannitol, C:N ratio, carbon and nitrogen content were measured at several time points.

All physiological and biochemical parameters tested were significantly affected by low temperature. Chlorophyll a, accessory pigment pool and F_v/F_m were significantly lower at 0 °C, while the de-epoxidation state was increased at both 0 °C and 8 °C. In contrast, pigment content and Fv/Fm were to a large extent irresponsive to salinity; however, mannitol content and growth decreased with decreased salinity.

This study reveals a significant impact of temperature and salinity variation on *S. latissima* performance. Negative effects of salinity are exacerbated at low temperatures. We report for the first time in *S. latissima* an increase in the osmolyte mannitol in response to low temperature that has ecological and economic implications.

Introduction

Kelps (order Laminariales, class Phaeophyceae) are important primary producers and ecosystem engineers in coastal ecosystems (Dayton, 1985; Bartsch et al., 2008). Moreover, kelps are rich in bioactive compounds with applications in food (Wells et al., 2017), feed (Correa et al., 2016), energy (Fernand et al., 2017), agrochemicals and pharmaceutical industries (Holdt and Kraan, 2011). Among those, certain pigments, such as carotenoids, are natural colorants and antioxidants with applications in human health (Wells et al., 2017). Moreover, the sugar alcohol mannitol has application in cosmetics and pharmaceuticals. In Phaeophyceae, besides its role as storage compound (Scheschonk et al., 2019), mannitol also has a function in osmotic adjustment and therefore changes in concentration follow variation in the salinity regime (Iwamoto and Shiraiwa, 2005; Gylle et al., 2009). *Saccharina latissima* is a kelp species common 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). Beyond its ecological relevance as primary producer and ecosystem engineer, interest in the cultivation of *S. latissima* has been rising given the several emergent industrial applications (e.g. Sanderson et al., 2012; Boderskov et al., 2016; Lopez-Contreras et al., 2016).

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 kelps by season and geographical location (Fernandes et al., 2016; Schmid et al., 2017). However, links to the specific abiotic factors and their interactions driving changes in biochemical composition remain poorly explored. Laboratory experiments with cultivated material limit the confounding effects of multivariate abiotic factors at play in the field (Davison, 1987). Moreover, laboratory experiments with multifactorial designs allow the exploration of interactive effects that can be related to field conditions.

Temperature is a major factor driving distributional ranges both at global and local scale by modulating survival, growth and reproduction of macroalgae (Lüning, 1990a; 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 drives significant modulation at the physiological and biochemical level in seaweeds, with effects in growth and survival (Gordillo et al., 2002; Spurkland and Iken, 2011); for example salinity effects in photochemistry have been reported (Scherner et al., 2013). Subtidal seaweeds, such as S. latissima, are expected to inhabit a more stable saline environment in comparison with intertidal species. Therefore, they are expected to be less tolerant to salinity changes than seaweeds living upper in the shore (Kirst, 1990; Gordillo et al., 2002). However, subtidal kelps are locally and intermittently subjected to hyposalinity driven by precipitation events, tidal ranges and freshwater input (Lüning, 1990b; 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 (Druehl, 1967; Druehl and Hsiao, 1977). Hence, *S. latissima* has been reported to survive salinities of S_A 10 and to grow from S_A 13 on (Karsten, 2007; Spurkland and Iken, 2011). Nevertheless, the interaction of temperature and salinity effects on macroalgae performance has been rarely investigated. Understanding acclimation strategies to both temperature and salinity changes will inform conservation practices and ecological consequences of local environmental changes.

Saccharina latissima has been traditionally harvested and it is recently also commercially cultivated in Brittany, France (Mesnildrey et al., 2012; Bernard et al., 2019). *S. latissima* in Roscoff, Brittany is exposed to high tidal coefficients (Gévaert et al., 2003) and low tides might lead to exposure to low salinity (Lüning, 1990a). 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 physiological and biochemical responses of the sugar kelp *S. latissima* to variations in temperature and salinity juvenile sporophytes were exposed under controlled laboratory conditions and samples were taken at several time points within 18 days. Hence, this study explores the time-dependent acclimation mechanisms to temperature and salinity in *S. latissima* that underline the species wide distribution.

Material and Methods

Algal material and experimental design

Young sporophytes of *S. latissima* were raised from stock cultures of uniparental male and female gametophytes (#3 2013 Roscoff) at the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI, Bremerhaven, Germany) as described by Heinrich et al. (2012). Vegetative gametophytes were isolated from spores of one fertile sporophyte collected at Roscoff (48° 43' 39" N, 3° 59' 13.2" W; Brittany, France). Sporophytes were cultivated in 5 L glass beakers at 8 °C under a photon fluence rate of 20 µmol photons m-2 s-1 of photosynthetically active radiation (PAR) (Mitras Lightbar Daylight 150, GHL, Germany) with a 18h-light:6h-dark photoperiod in an environmentally controlled room. Algae were cultivated for three months in sterile seawater enriched with Provasoli (Starr and Zeikus, 1993) with an absolute salinity (S_A) of approx. 30 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 exposed to the temperatures 0 °C, 8 °C and 15 °C for seven days (n = 5). After seven days, sporophytes were exposed to a low salinity treatment (S_A 20) and a control one (S_A 30) by each temperature for eleven days in a total of eighteen experimental days.

Growth and maximal quantum yield of photosystem II (*Fv/F*m)

During the experimental phase (18 days) fresh weight (n = 3) and maximal quantum yield of photosystem II (*Fv/F*m, n = 5) were measured twice a week. Maximal quantum yield of photosystem II (*Fv/F*m) was measured with an Imaging PAM (Pulse Amplitude Fluorometer; Walz, Effeltrich, Germany) after 10 min dark acclimation (n = 5). *Fv/F*m values measured with imaging PAM are often slightly lower than with other PAM instruments (Nielsen and Nielsen, 2008), therefore initial values of *Fv/F*m (~ 0.5) were slightly lower than the range considered healthy for brown algae (~ 0.7) (Dring et al., 1996; Hanelt, 1998). Fresh weight was measured by an analytical digital balance after blotting (Sartorius LA310S) (n = 3). Growth was calculated as percentage of initial to account for variability among replicates at the start of the experiment. Area was measured at the start of the experiment using ImageJ based on pictures taken (n = 5) (Schneider et al., 2012).

Biochemical parameters

Samples for biochemical analysis (pigments, mannitol, C and N content) 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, Osterode am Harz, Germany) for 24 hours at 1 mbar and -55 °C. Mannitol content was measured on days 8, 11 and 18. For the extraction, lyophilized and homogenized 8-10 mg samples were incubated with 1 mL aqueous ethanol (70%, v/v) for 3-4 hours in a water bath at 70 °C. After centrifugation (5 min; 13,000 rpm), 800 µL of the supernatant was transferred to a new Eppendorf tube and evaporated to dryness with a Speed Vac (Alpha 1-4 LSC plus and RVC 2-25 CDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Once samples were dried, we added 800 µL of HPLC grade water to the dried samples and re-dissolved in an ultra-

sonic bath and frequent vortexing. When dissolved, samples were centrifuged again (5 min; 13,000 rpm). 750 µL of the supernatant was transferred to vials for analysis with a HPLC Agilent Technologies system (1200 Series, Santa Clara, California, USA) with an Aminex Fast Carbohydrate Analysis Column HPAP (100x7.8 mm, 9 µm, BioRad, Munich, Germany), protected by a guard cartridge (Phenomenex, Carbo-Pb-2+ 4 x 3.00 mm I.D., Aschaffenburg, Germany) with 100% Milli-Q water as a mobile phase, following the method of Karsten et al. (1991a). D(-)-mannitol standards ($C_6H_{14}O_6$, Roth) of 1, 6 and 10 mmol were used for calibration. A RI-Detector (35 °C) and the software 'ChemStation for LC 3D systems' (Agilent Technologies, Waldbronn, Germany) were used to analyze the samples. Mannitol contents were calculated in µmol g-1 dry weight (DW).

C:N ratio, total carbon (C) and total nitrogen (N) contents were analyzed following Graiff et al. (2015) on samples taken on days 8, 11 and 18. 2-3 mg of lyophilized and ground samples (n = 4) were weighed and packed into tin cartridges (6 x 6 x 12 mm) and combusted at 950 °C. The content of C and N were quantified automatically in an elemental analyzer (Vario EL III, Elementar, Langenselbold, Germany). As standard acetanilide (C_8H_9NO) was used (Verardo et al. 1990). Total C and total N content were calculated in mg g-1 dry weight (DW). The C:N ratio was calculated based on these results.

Pigment content was determined using a high performance liquid chromatography (HPLC) following the protocol by Koch et al. (2016), on days 8 and 18 (n = 5). 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 $DPS = ((Z+0.5A))/(V+A+Z)$ as described in Colombo-Pallotta et al. (2006).

Statistical analysis

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. When data complied with the assumptions, a two-way ANOVA was applied to pigment content on days 8 and 18 and to F_v/F_m with temperature and salinity as fixed factors. A repeated measures ANOVA was applied to fresh weight with between-subjects effects of temperature and salinity and within-subjects effect of time. A three-way ANOVA for the effect of time, temperature and salinity was applied to mannitol, C:N ratio, total carbon and total nitrogen. When the factor time was not significant, a two-way ANOVA for the effect of temperature and salinity was applied to mannitol, C:N, total carbon and total nitrogen for each time point measured. 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. Considering the high standard deviation observed for some biochemical parameters, we performed a Grubb's test to detect outliers (Grubbs, 1969) through the webpage GraphPad (https://www.graphpad.

com/quickcalcs/grubbs1/). For subsequent analysis these outlier values were excluded.

Results

Maximal quantum yield of photosystem II (*F*v/*F*m)
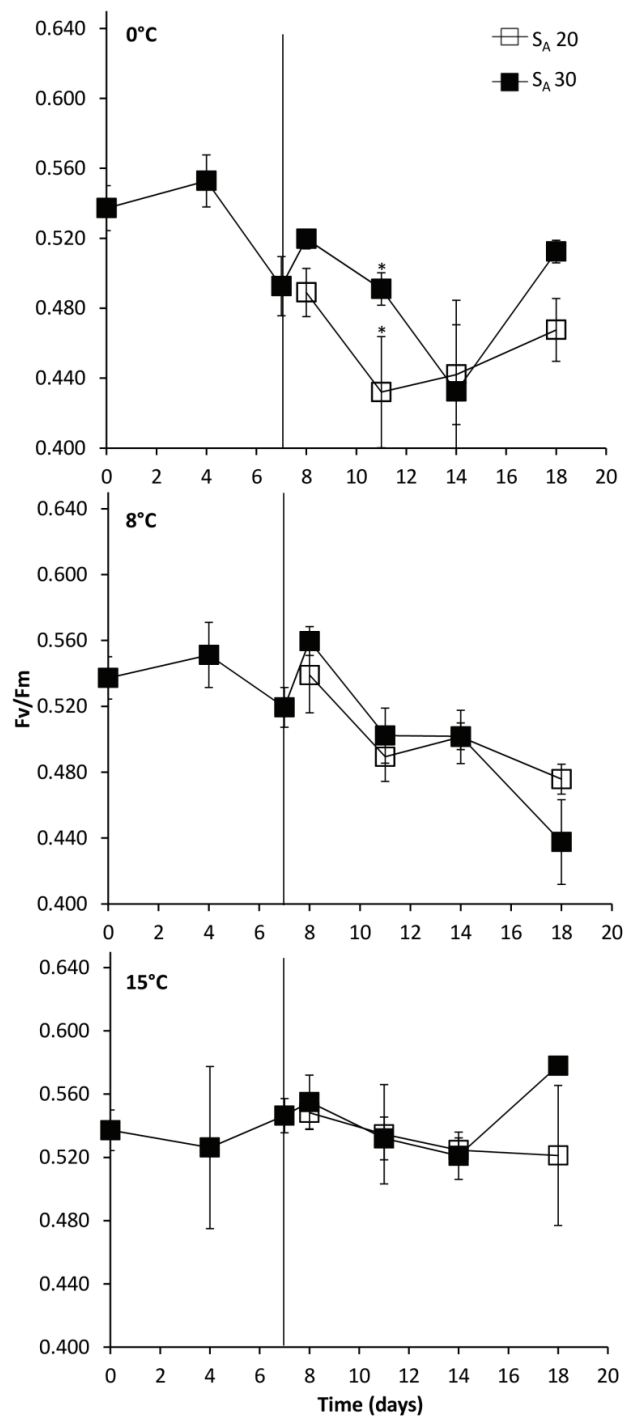
Figure 1 Maximal quantum yield of photosystem II (*F*v/*F*m) of *S. latissima* from Roscoff after 0, 4, 7, 8, 11, 14 and 18 days of experiment at 0 °C, 8 °C and 15 °C (mean ± SD; n = 5). Black boxes mark S_A 30, white boxes S_A 20. On the left, temperature acclimation phase – 7 days at 0 °C, 8 °C, 15 °C and control salinity S_A 30; and on the right, salinity x temperature acclimation –exposure to low salinity (S_A 20) started on day 7, while control samples were maintained at SA 30 within 0 °C, 8 °C, 15 °C for 11 days. Asterisks stand for the significant differences between salinities ($p < 0.05$).

*F*v/*F*m was not significantly affected by temperature at day 4 (p = 0.826) (Figure 1, Table S1). On day 7, *F*v/*F*m was significantly higher at 15 °C than at 8 °C and higher at 8 °C than at 0 °C (p = 0.000).

Temperature had a significant impact on *F*v/*F*m on each measurement day during the 11 days of temperature and salinity acclimation (Figure 1, Table S2). On day 8, *F*v/*F*m values were significantly lower at 0 °C) than at 8 °C and at 15 °C (p = 0.000). On day 11, *F*v/*F*m was significantly affected by temperature (p = 0.000), salinity (p = 0.007), and the interaction of temperature and salinity (p = 0.010). *F*v/*F*m was significantly higher at SA 30 than SA 20 at 0 °C, but not at 8 °C and 15 °C. On days 11 and 14, Fv/Fm values were significantly lower at 0 °C than at 8 °C and at 15 °C. On day 18, *F*v/*F*m measured at 15 °C was significantly higher than at 8 °C.

Growth
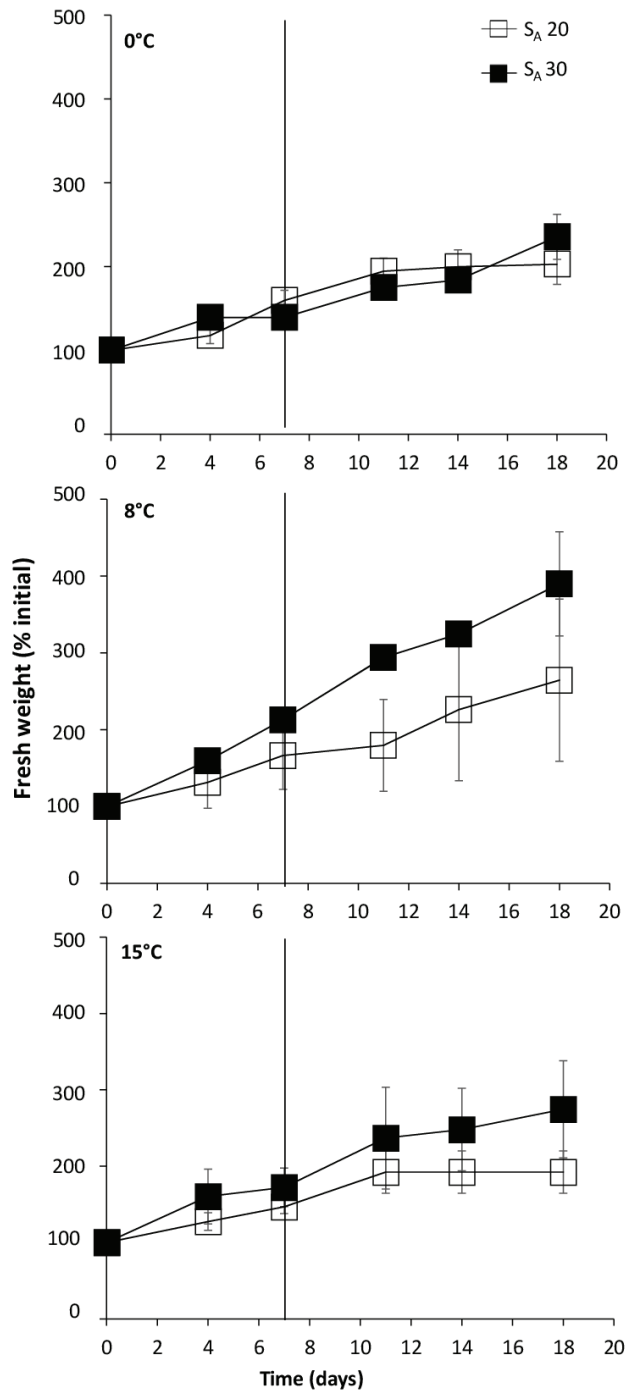
Figure 2. Fresh weight of *S. latissima* from Roscoff after 0, 4, 7, 11, 14 and 18 days of experiment at 0 °C, 8 °C and 15 °C (% of initial weights; mean ± SD; n = 3). Black boxes mark S_A 30, white boxes S_A 20. On the left, temperature acclimation phase – 7 days at 0 °C, 8 °C, 15 °C and control salinity S_A 30; and on the right, salinity x temperature acclimation –exposure to low salinity (S_A 20) started on day 7, while control samples were maintained at S_A 30 within 0°C, 8°C, 15°C for 11 days.

During temperature acclimation, growth (as percentage of initial) was significantly affected by time (p = 0.000), the interaction of time and temperature (p = 0.018), but not by temperature alone (p = 0.165) (Table S3). Fresh weight was higher after 7 days than at day 4. After 7 days, fresh weight at 8

°C was higher than at 0 °C and non-significant (p = 0.062, pairwise comparisons, Bonferroni adjustment) (Figure 2).

During salinity acclimation, growth was significantly affected by time (p = 0.000), the interaction of time and temperature (p = 0.014), the interaction of time and salinity (p = 0.011), temperature (p = 0.025) and salinity (p = 0.026) (Table S4). Fresh weight was higher at S_A 30 than at S_A 20 at 11 days and 18 days of the experiment. Growth was significantly lower at 0 °C than at 8 °C. Fresh weight at 0 °C and 15 °C significantly increase from day 7 to day 11, but subsequent increase in growth was not significant. Growth at 8 °C increased significantly between days 7 and 11, 11 and 14 and 14 and 18 (Figure 2).

Pigments

Day 8
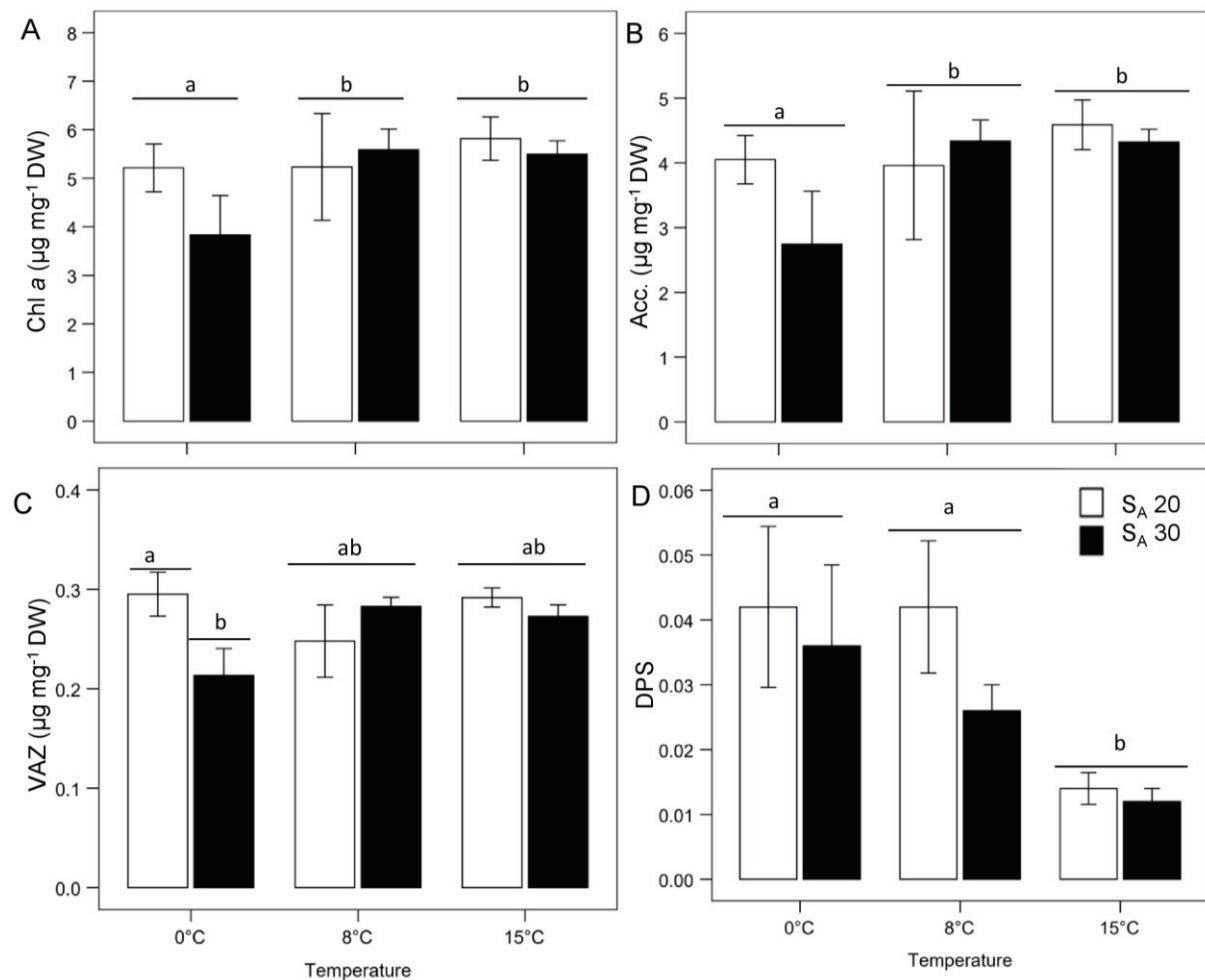
Figure 3 Chl a content (A), Acc. (B), VAZ (C) (µg mg−1 DW; mean ± SD; n = 5) and DPS (D) (mean ± SD; n = 5) of *S. latissima* from Roscoff after exposure to two salinity conditions (S_A 20, 30) within three temperatures (0 °C, 8 °C, 15 °C) on day 8. Black boxes mark S_A 30, white boxes S_A 20. Significant differences between treatments are shown by different letters (p < 0.05). Significant differences were identified for Chl a and Acc. after exponential transformation.

Chl a (chlorophyll a), Acc., VAZ and DPS were all significantly affected by temperature at day 8 (24h of salinity exposure), except for VAZ which was only affected by the interaction of temperature and

salinity (Table S5). In turn, salinity did not significantly affected pigments except for the above mentioned interaction term for VAZ. VAZ was significantly higher at S_A 20 than S_A 30 at 0 °C (Figure 3). DPS was significantly lower at 15 °C than at 0 °C and 8 °C. Chlorophyll a and accessory pigments were significantly lower at 0 °C than at 8 °C and 15 °C.

Day 18
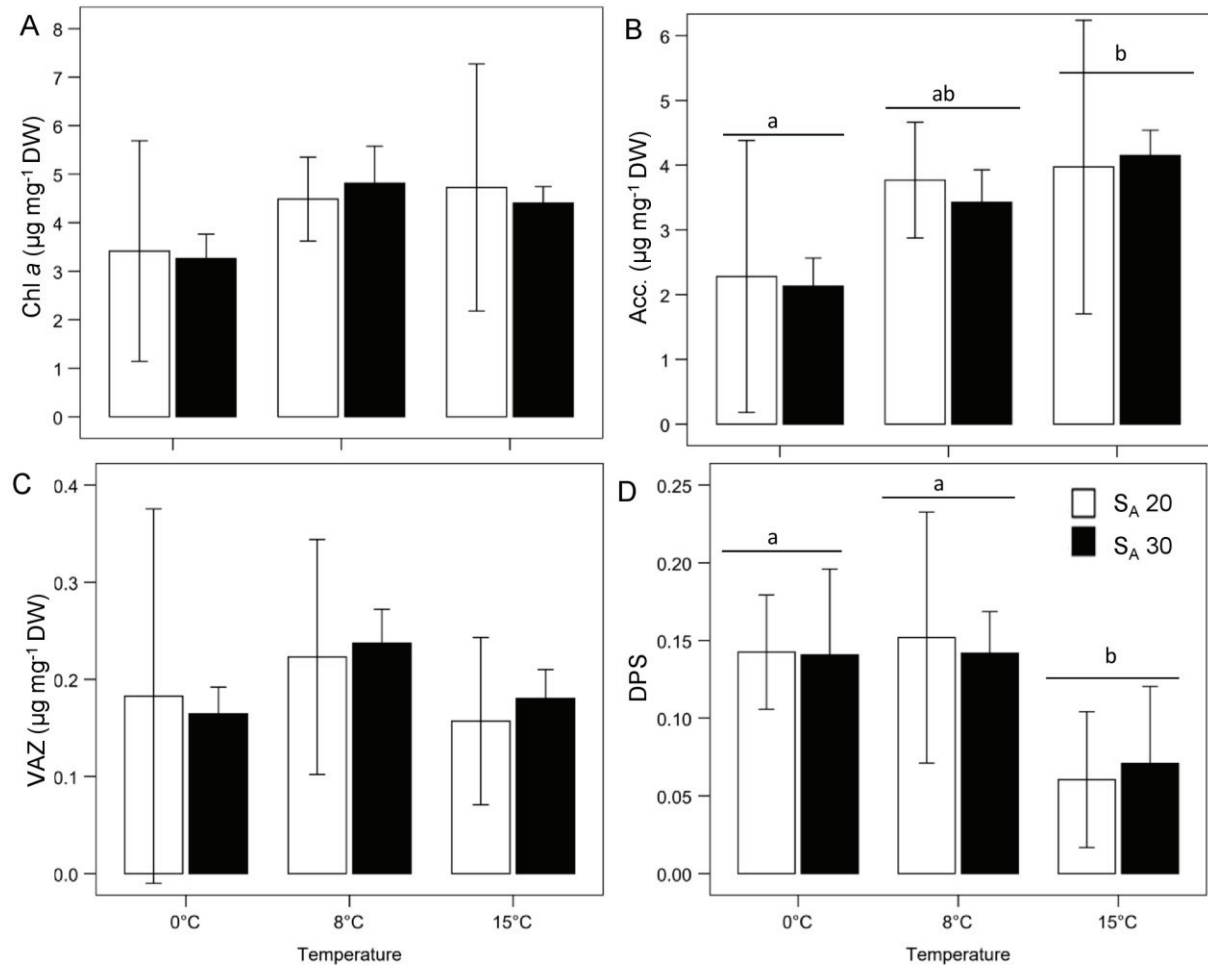
Figure 4 Chl a content (A), Acc. (B), VAZ (C) (µg mg−1 DW) and DPS (D) (mean ± SD; n = 5) of *S. latissima* from Roscoff after exposure to two salinity conditions (S_A 20, 30) within three temperatures (0 °C, 8 °C, 15 °C) on day 18. Significant differences between treatments are depicted by different letters (p < 0.05).

Acc. and DPS were significantly affected by temperature at day 18 (11 days of salinity acclimation), but not chl a and VAZ (Table S6, Figure 4). In turn, salinity did not significantly affected pigments at day 18. DPS was significantly lower at 15 °C than at 0 °C and 8 °C. Acc. content was significantly higher at 15 °C than at 0 °C.

Mannitol
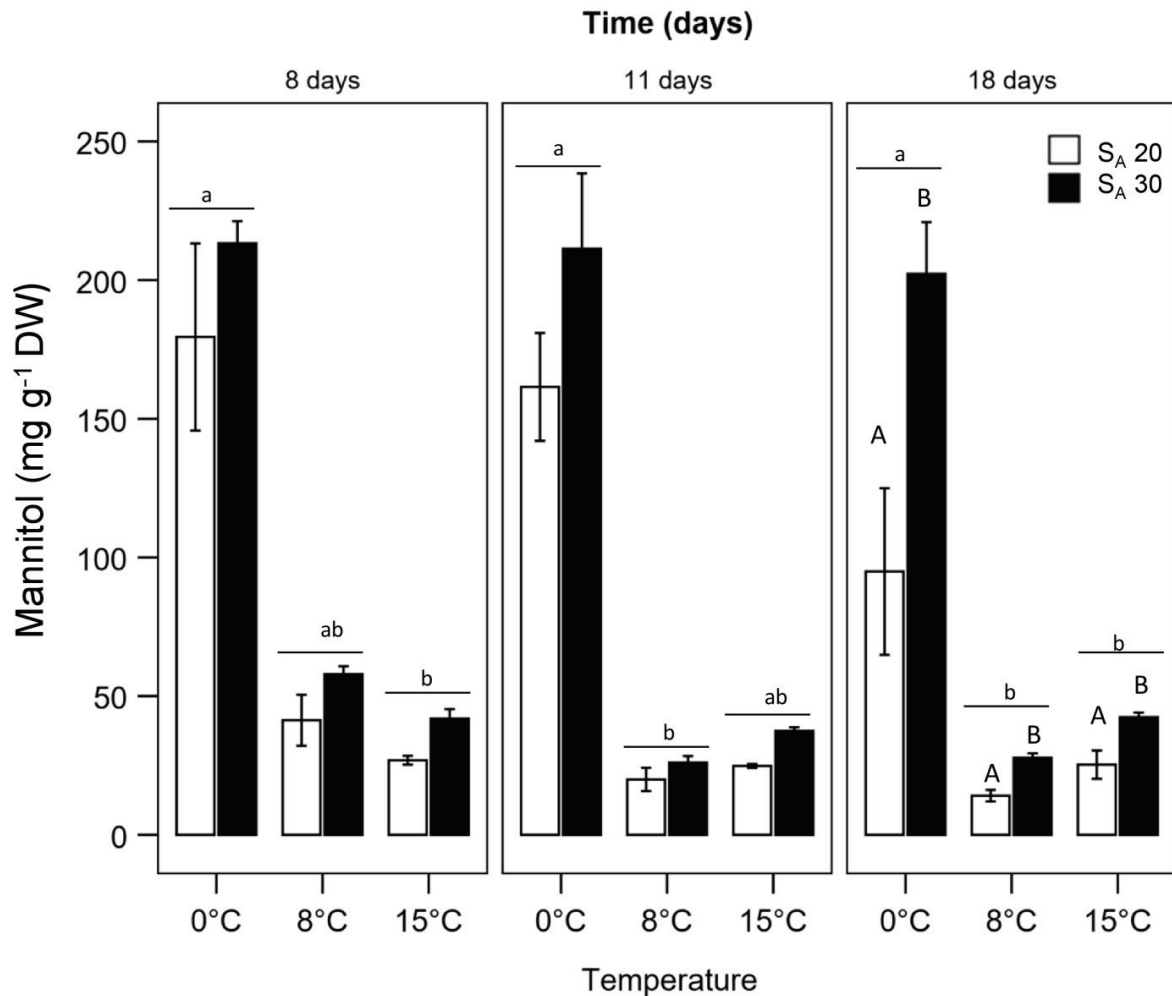
Figure 5 Mannitol concentration (mg g−1 DW; mean ± SD; n = 3) of *S. latissima* from Roscoff after exposure to two salinity conditions (S_A 20, 30) within three temperatures (0 °C, 8 °C, 15 °C) on days 8, 11 and 18. Different lower case letters indicate significant differences between temperatures ($p < 0.05$). Different upper case letters indicate significant differences between salinities ($p < 0.05$). Significant differences were identified at day 18 after log10 transformation.

Time did not have a significant effect on mannitol concentrations ($p = 0.155$; Figure 5, Table S7). Overall, mannitol content was significantly lower at low salinity (S_A 20) than at the control - S_A 30 ($p = 0.010$). Overall, mannitol concentration at 0 °C was significantly higher than at 8 °C and 15 °C ($p = 0.000$). On day 8, mannitol content at 0 °C was significantly higher than at 15 °C ($p = 0.001$) while on day 11, significant differences between 0 °C and 8 °C were observed ($p = 0.001$). On day 18, mannitol content was significantly lower at S_A 20 than at S_A 30 ($p = 0.001$), but significance was not detected on days 8 and 11. On day 18, mannitol content at 0 °C was significantly higher than both 8 °C and 15 °C ($p = 0.000$).

C:N, total C and total N content
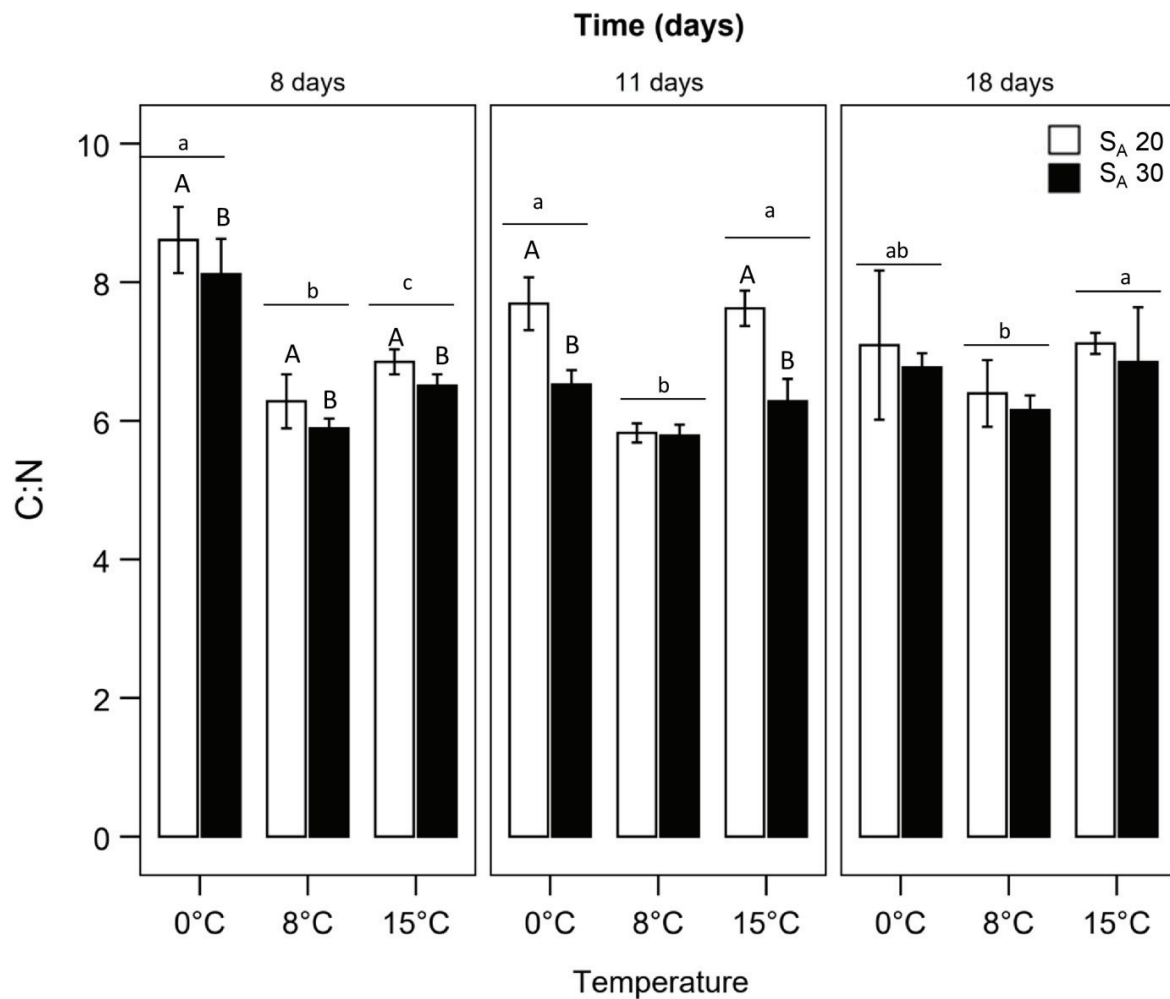
Figure 6 C:N ratio (mean ± SD; n = 4) of *S. latissima* from Roscoff after exposure to two salinity conditions (S_A 20, 30) within three temperatures (0 °C, 8 °C, 15 °C) on days 8, 11 and 18. Different lower case letters indicate significant differences between temperatures ($p < 0.05$). Different upper case letters indicate significant differences between salinities ($p < 0.05$). Significant differences were identified at day 8 and 11 after log10 transformation.

On day 8 and day 11, the C:N ratios were significantly affected by temperature and salinity (Figure 6, Table S8). On day 8, differences between the three temperatures were significant ($p = 0.000$). Additionally, the C:N ratios on day 8 were affected by salinity, resulting in higher C:N ratios at lower salinities ($p = 0.006$), but no temperature × salinity interaction was found. On day 11, the C:N ratio was significantly higher at 0 °C and 15 °C than at 8 °C ($p = 0.000$). Furthermore, interactions revealed significant differences between S_A 20 and SA 30 at both 0 °C and 15 °C ($p = 0.001$) on day 11. On day 18 significant differences were observed between 8 °C and 15 °C ($p = 0.020$). Day 18 could not be tested for interactions.

Figure 7 Carbon concentration (mg g−1 DW; mean ± SD; n = 4) of *S. latissima* from Roscoff after exposure to two salinity conditions (S_A 20, 30) within three temperatures (0 °C, 8 °C, 15 °C) on 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).

The total carbon (C) content varied between the different treatments (Figure 7, Table S8). After 24h of salinity stress (day 8), significant differences were detected between temperature and salinity. At all temperatures the samples at S_A 20 had higher C concentrations than at S_A 30 (p = 0.017). Additionally, samples exposed to 0 °C contained significantly more C than 8 °C (p = 0.001). On day 11, the C content differed between 8 °C and 15 °C, as well as 8°C and 0 °C (p = 0.001), while 0 °C and 15 °C do not differ significantly. There was no significant decrease or increase in C detected over time. Nevertheless, C concentrations varied slightly between treatments during the experiment. Hence, on day 18 significant differences between treatments were no longer detected.
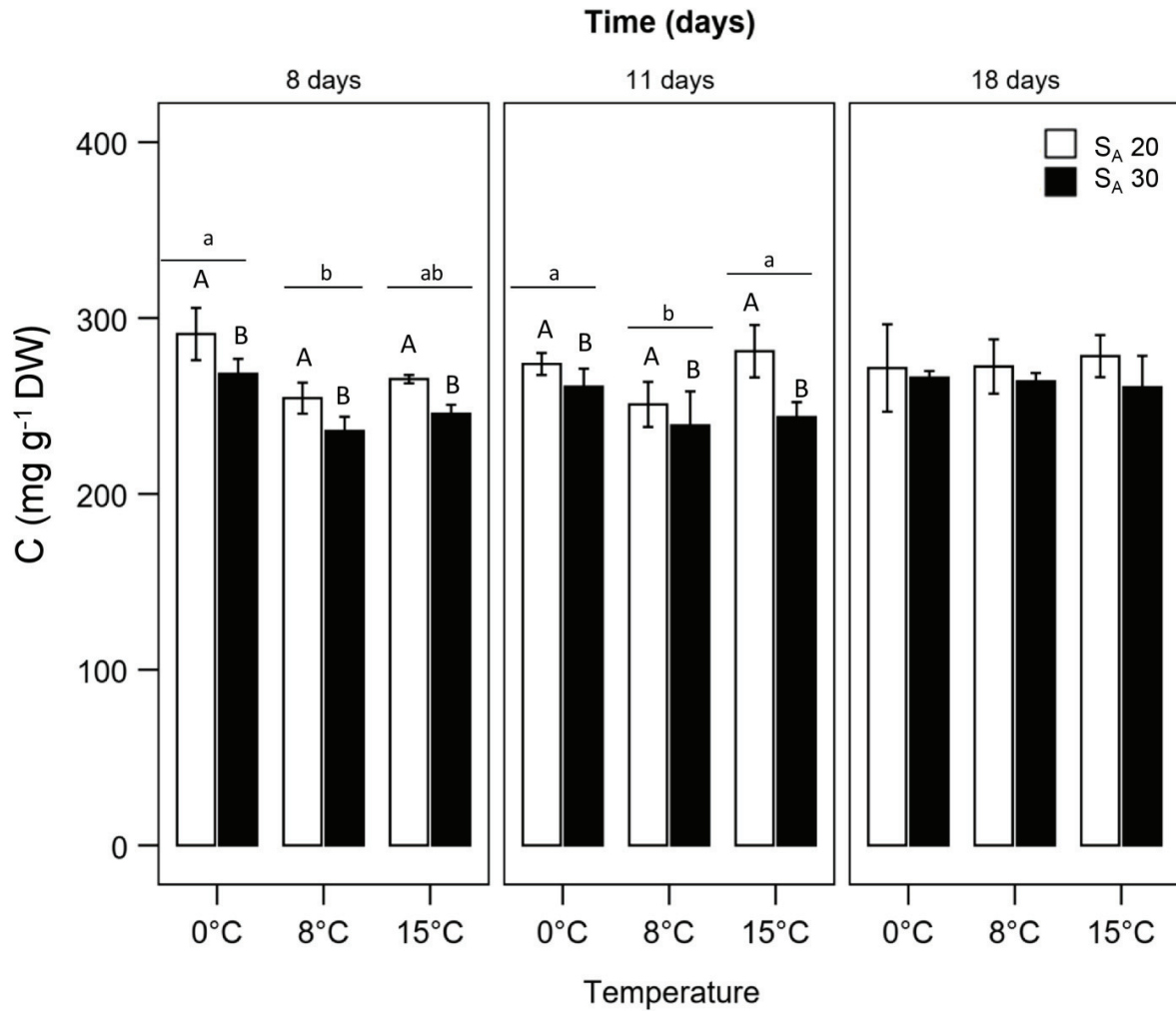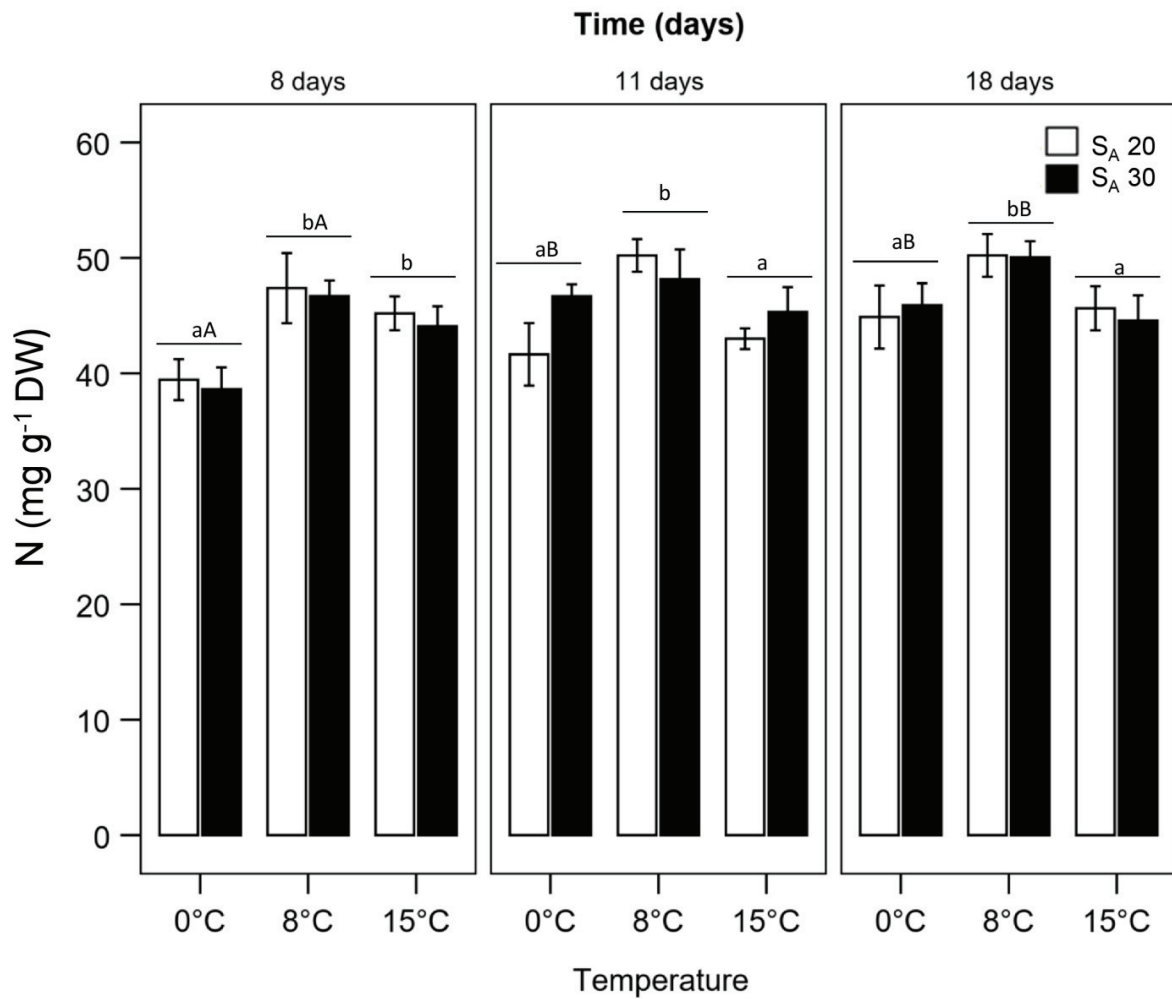
Figure 8 Nitrogen concentration (mg g−1 DW; mean ± SD; n = 4) of *S. latissima* from Roscoff after exposure to two salinity conditions (S$_A$ 20, 30) within three temperatures (0 °C, 8 °C, 15 °C) across time (days 8, 11 and 18). Different lower case letters indicate significant differences between temperatures (p < 0.05). Different upper case letters indicate significant differences between time points (p < 0.05).

There was a significant effect of time on the total nitrogen (N) content (p = 0.000; Figure 8, Table S8). Between day 8 and day 11, as well as day 8 and day 18 the N concentration increased significantly. Salinity did not significantly impact the N content (p = 0.575). There was a significant effect of temperature (p = 0.000) and of the interaction between time and temperature (p = 0.001) on N content. Significant differences across temperatures in the total Nitrogen (N) content were observed on day 8 (Figure 8). The 0 °C treatments featured significantly lower N concentrations compared to 8 °C and 15 °C (p = 0.000). On day 11 and day 18, N at 0 °C differed from the content at 8 °C (p < 0.001), but not from 15 °C. N content at 0 °C significantly increased from day 8 to day 11 and from day 8 to day 18, while N content at 8 °C increased significantly from day 8 to day 18.

Comparison with sporophytes from Spitsbergen

The data presented in this Chapter for sporophytes originated in Roscoff can be compared with the results described in Chapter 3 (Li et al. 2019) as we followed the same approach in both Chapters.

Fv/Fm

Algae from both geographical origins showed initial values of *Fv/Fm* within the range 0.5 to 0.6. During the experiment, overall higher temperatures lead to higher *Fv/Fm* values in sporophytes from both locations, Roscoff and Spitsbergen (Chapter 3 – Figure 2 and this Chapter Figure 1). Hyposaline conditions resulted in a reduction in *Fv/Fm* values in algae from both origins, however significant differences arose at different time points. On day 18 *Fv/Fm* values were significant lower at 15 °C S_A 20 than at 15 °C S_A 30 in Spitsbergen samples while on day 18 only temperature had a significant effect on *Fv/Fm* measured in Roscoff algae. In turn, on day 11 a significant effect of temperature, salinity and the interaction of both on *Fv/Fm* values of sporophytes from Roscoff was observed.

Growth

Growth (measured as percentage of initial) was higher in Spitsbergen sporophytes than Roscoff ones. On the last day of the salinity x temperature experiment, algae from Spitsbergen reached average 800% of the initial weight for the 15 °C control salinity treatment (Chapter 3 – Figure 1), while algae from Roscoff only reached 400% for the 8 °C control salinity treatment (this Chapter – Figure 2). Hyposalinity resulted in a decrease in growth in both Roscoff and Spitsbergen algae. However, while in Roscoff a significant decrease in weight was observed already at day 11, in Spitsbergen the reduction was only significant on day 18. Moreover, the hyposalinity-driven reduction in growth was less pronounced in Roscoff than in Spitsbergen. While in Spitsbergen mean fresh weight at day 18 was 427% of initial at S_A 20 and 648% at S_A 30, in Roscoff it was 230% and 300% respectively.

Pigments

On day 8, temperature did not affect chl a and DPS in algae from Spitsbergen (Chapter 3 – Figure 3) but it affected algae from Roscoff. In sporophytes from Roscoff, chl a content was significantly lower at 0 °C than 8 °C and 15 °C, while DPS was significantly lower at 15 °C than at 0 °C and 8 °C. Accessory pigment pool increased with temperature in sporophytes from both locations. Salinity significantly affected VAZ pool in algae from both sites. In sporophytes from Spitsbergen the overall VAZ content was higher in the S_A 20 treatments compared to the control and no significant interaction between temperature and salinity was observed. In turn, in sporophytes from Roscoff, VAZ was only significantly higher in S_A 20 at 0 °C.

On day 18, chl a content remained constant across treatments and across geographical origin (chapter 3 – Figure S3 and this Chapter Figure 4). While accessory pigments, VAZ and DPS, were affected by salinity or the interaction of temperature and salinity in algae from Spitsbergen, there was no effect on algae from Roscoff. Moreover, Acc. was significantly higher at 15 °C than 0 °C in algae from Spitsbergen and Roscoff. While DPS was significantly higher at 0 °C than at higher temperatures in sporophytes from Spitsbergen, in algae from Roscoff DPS measured at 0 °C and 8 °C was not significantly different.

Mannitol, C:N ratio, C and N content were only measured in sporophytes from Roscoff.

Differences driven by geographical variation

In order to assess differences in responses between algae from Roscoff and Spitsbergen, we tested for the effect of origin in the initial values (day 0) before the start of the experiment. For all the physiological parameters measured (Fv/Fm, pigments and area) there was a significant difference between initial values of algae from Roscoff and Spitsbergen (Table S9). Initial absolute values of area, photoprotective/light harvesting pigments, VAZ/chl a and Fv/Fm were significantly higher in Roscoff than Spitsbergen. Initial values of chl a were significantly higher in Spitsbergen than Roscoff (Figure 9).
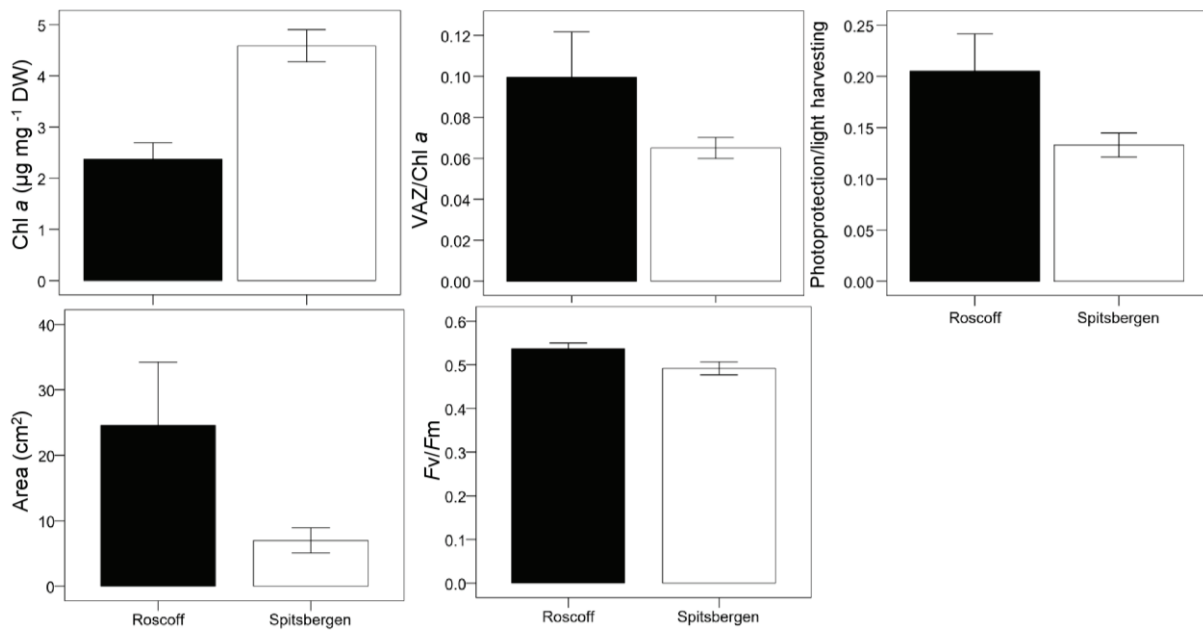
Figure 9 Chl a (μg mg -1), VAZ:chl a, ratio of photoprotection/ light harvesting ((VAZ + ß-carotin)/ (fucoxanthin + chl. c2)), area (cm2) and Fv/Fm measured at the beginning of the experiment (day 0) in algae from Roscoff and Spitsbergen. Algae were cultivated at 8 °C S_A 30 for three months. Results are given as mean ± SD.

Discussion

Salinity significantly affected mannitol content and growth, which reveals a relevant impact on physiology. In turn, salinity had little impact on photo-ecophysiological parameters measured. The low temperature tested here (0 °C) significantly affected the physiological and biochemical response variables under investigation (Fv/Fm, growth, pigment, mannitol, C and N content and C:N ratio). In turn, little differences were observed between the temperatures 8 °C and 15 °C. Furthermore, a stronger impact was detected after short-term stress treatments (day 8). This is in accordance to the optimal temperature range for *S. latissima* being between 10-15 °C (Fortes and Lüning, 1980; Bolton and Lüning, 1982). Even though 8 °C is slightly lower than this range, algae responded very similarly to both 8 °C and 15 °C. In turn, exposure to 0 °C led to lower Fv/Fm values at several time points and lower growth. In addition, the content of pigments such as chlorophyll a, accessory pigments was

lower at 0 °C on day 8 (accessory pigment pool also on day 18). This response indicates compromised fitness at low temperature.

Pigment content measured in young cultivated sporophytes of *S. latissima* in this study fall within the range measured by Olischläger et al. (2017) for the same species at similar life stage. In contrast, pigment content measured in this study is one order of magnitude higher than reported in field adult sporophytes in Denmark (Boderskov et al., 2016; Nielsen et al., 2016). These differences suggest a possible effect of age (young vs. adult) or origin (cultivated vs. field samples) in pigment content, warranting further investigation on the topic. We observed a decrease of the de-epoxidation state (DPS) at 15 °C when compared to 0 °C and 8 °C in algae from Roscoff. In algae from Spitsbergen, DPS did not differ significantly at day 8, but on day 18 was significantly higher at 0 °C (Li et al., 2019). Similarly, Olischläger et al. (2017) observed a decrease in DPS at higher temperatures for sporophytes from Spitsbergen (4 °C to 10 °C) and sporophytes from Helgoland (10 °C to 17 °C) after 18 days of exposure to a combination of temperature and pCO_2 levels. The xanthophyll cycle pigment pool (VAZ) was unaffected by temperature in this study. In turn, an increase at 15 °C in algae from Spitsbergen was observed (Li et al., 2019). Different modulation of VAZ content between algae originating in different locations (Spitsbergen and Helgoland) has already been observed (Olischläger et al., 2017). VAZ was also significantly affected by salinity. Higher VAZ content was measured at S_A 20 at 0 °C on day 8. In algae from Spitsbergen this effect was more extensive as VAZ was higher at S_A 20 at all temperatures (Li et al., 2019). The xanthophyll cycle is a component of stress response of plants, brown and green seaweeds (Goss and Jakob, 2010). High DPS values provide protection from photo-oxidative damage by energy dissipation and therefore it is expected to be higher under stressful conditions, such as the low temperature applied in this study (Müller et al., 2001; Fernández-Marín et al., 2011).

Mannitol concentrations were considerably higher at 0 °C than at 8 °C and 15 °C across the experimental period. 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 associated, among other mechanisms, with sugar alcohol content (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 (Zubia et al., 2008). Similarly, in a marine ecotype of *Fucus vesiculosus*, mannitol content was higher at 0 °C than at 10 °C, although there were no significant changes in the brackish ecotype (Gylle et al., 2009). Using mannitol as cryoprotectant seems to be a fast response to cold shock, explaining the high mannitol concentrations on day 8 at 0 °C and low salinities. The role of mannitol as osmolyte and compatible solute has already been described in several studies (e.g. Kirst, 1990; Eggert et al., 2007, Diehl et al. submitted). As expected, hypoosmotic conditions led to a decrease in mannitol content in this study to prevent water inflow into the cell. Osmotic acclimation is a two phased process. The first step is a fast change in turgor pressure and changes in cellular concentrations of potassium, sodium and chloride (Karsten et al., 1991b), which can last between minutes to hours in macroalgae (Kirst, 1990). Adjustment in osmolyte concentration has been described as the second stage, being a slow and long-term response to changing osmotic conditions, taking up to a few days and being energy demanding (Kirst, 1990). We measured differences in mannitol content already after a 24h exposure (yet not significantly) to low salinities, similar to was reported for *Fucus vesiculosus* (Gylle et al., 2009) and *Pilayella littoralis*

(Reed et al., 1985). With longer exposure to low salinities, the mannitol concentration decreased significantly, especially at 0 °C. Hence, cold shock is exceeding salinity stress. On day 18, the mannitol concentration at S_A 20 was significantly lower than at S_A 30, bearing out that adjustment in osmolyte concentrations is a long-term process. While there was no significant difference between 8 °C and 15 °C on day 18, the trend shows increasing mannitol concentrations at 15 °C. Since mannitol is the main photosynthetic product of brown algae, it might be positively affected by temperature increase (Ji et al., 2016). Gene expression revealed that the mannitol-1-P dehydrogenase gene was not significantly downregulated under hyposaline stress, while choline dehydrogenase, enzyme with a role in biosynthesis of glycine betaine, was (Li et al., 2019). In further studies, glycine betaine should be analysed to shed light on its role on acclimation to salinity stress.

The C:N ratio is commonly used as an indicator for N limitation. In a study from 1983, Atkinson and Smith reported that temperate and tropical benthic macroalgae feature a mean C:N ratio of 20. They considered a C:N ratio of 10 to be very low, reflecting sufficient N supply. The samples measured in this study have a C:N ratio of approx. 6-10, meaning that the algae did not suffer from N limitation in any experimental condition. Considering the study of Peters et al. (2005) who detected C:N ratios below 10 only in red and green macroalgae and the results of Scheschonk et al. (2019), *S. latissima* in this study has considerably lower C:N ratios than reported before in other Phaeophyceae. Several studies report increasing C:N ratios due to heat stress on brown macroalgae (e.g. Gordillo et al., 2006; Graiff et al., 2015). In this study, C:N ratio was significantly higher at 0 °C on day 8, however on day 18 mean values of C:N measured at three temperatures were more similar, with significantly higher values at 15 °C than 8 °C. Conclusively, results suggest that *S. latissima* is negatively affected by the low temperature of 0°C, which can also be seen in the results of mannitol content and F_v/F_m. However, sufficient N uptake was still ensured as the C:N ratio remained below 10. Furthermore, samples kept at the control temperature of 8 °C showed significantly lower C:N ratios than 0 °C and 15 °C on days 11 and 18, indicating that short-term temperature changes are stressful for *S. latissima*.

Carbon assimilation and utilization can be affected by tissue structure, photosynthetic activity or growth (Gómez and Wiencke, 1998; Gévaert et al., 2001; Peters et al., 2005). However, the significant variations in total C content detected in this study cannot be explained neither by changes in F_v/F_m nor growth. Contrarily to previous reports, the C concentration at S_A 30 was significantly lower (while e.g. F_v/F_m is higher) at all temperatures compared to SA 20 on day 8. Lower salinity decreases the pH in seawater, related to a change in the CO2:H2CO3 ratio (Saraswat et al., 2011). Moreover, solubility of gases, such as CO_2 is also dependent on temperature. CO2 dissolves better in liquids at low temperatures (Dickson, 2010). More available CO_2 in the medium led to increasing C fixation in *Gracilaria lemaneiformis* (Chen et al., 2018). Therefore, a possible explanation for the higher content of C at 0°C and at SA 20 is that the resulting higher CO_2 concentrations in seawater reduces energy costs in carbon acquisition, since CO_2 concentrating mechanisms (CCMs) are not needed (Harley et al., 2012). Additionally, the impact of temperature on RuBisCO kinetics and on CCMs might play a role (Raven et al., 2002). C concentration appears to acclimate over the experimental period (although changes are not significant) resulting in no observable differences in C on day 18 in any treatment. Summarizing, significant changes in C content are mainly found after 24h in the hyposalinity treatment. This could indicate the synergistic effect of short-term low temperature/low salinity exposure.

Beside C content, also N content showed significant variation due to temperature changes, but not between the different salinities. *Saccharina latissima* growing in Arctic regions is reported to have a lower N content and higher C:N ratio (Henley and Dunton, 1995; Scheschonk et al., 2019, Diehl et al. submitted, Diehl et al. in prep) than recorded in this study. On day 8, the N concentration is significantly lower at 0 °C than at 8 °C and 15 °C, being indicative for a low-temperature response in S. latissima. The N uptake seems to be inhibited in *S. latissima* after short-term exposure to the lowest experimental temperature. Since the activity of enzymes is temperature dependent, decreases in N concentrations might be explained by decreased nitrate reductase activity or protein synthesis (Reay et al., 1999). Furthermore, there was a significant increase in N over time, while C content stabilized, which reveals that *S. latissima* can acclimate to low temperatures within eighteen days.

The responses of all biochemical and physiological parameter indicate compromised fitness and enhanced stress at very low temperatures, especially after short-term stress exposure in *Saccharina latissima* from Roscoff. Low salinity leads to physiological stress in S. latissima, which resulted in decreased photosynthetic efficiency and growth and increase in the osmolyte mannitol.

Differences driven by geographical variation

The existence of ecotypes has been described for *S. latissima* across its latitudinal range (Gerard and Du Bois, 1988; Gerard, 1988; Müller et al., 2008). An ecotype is defined to perform better at the local conditions than another ecotype from a distant population (Kawecki and Ebert, 2004). Therefore, we compared data gathered in this Chapter for Roscoff, Brittany to the data gathered on Chapter 3 (Li et al. 2019) for specimens from Spitsbergen, Arctic. Algae from both locations were cultivated at similar conditions from the gametophyte stage and were exposed to the same temperature and salinity levels during the experiment. In case of ecotypes, we expected algae from Spitsbergen to perform better at low temperature and low salinity (0 °C S_A 20), while algae from Roscoff would grow better at 15 °C and saline conditions. However, samples from Spitsbergen grew faster than algae from Roscoff at all treatments. 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 (at certain time points) and significantly impacted VAZ in both algae from Roscoff and Spitsbergen. Therefore, ecotypic differentiation cannot be implied from the results of biochemical and physiological parameters measured during this experiment. Nonetheless, we observed differences in several parameters at day 0, at the end of the cultivation phase (Figure 6). In addition, morphology differed considerably between algae from Roscoff and Spitsbergen. Sporophytes from Spitsbergen were narrower and longer while algae from Roscoff were wider and shorter. 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 Roscoff as chl a, DPS, Acc. and VAZ significantly changed in response to temperature after 24 hours (VAZ in response to the interaction of temperature and salinity), but chl a and DPS did not significantly change in algae from Spitsbergen. At day 18, only DPS and Acc. were significantly modulated in algae from both locations; however salinity effects were only significant in Spitsbergen. Moreover, significant decrease in growth due to low salinity was evident already at day 11 in algae from Roscoff but only at day 18 for algae from Spitsbergen. Furthermore, at the transcriptomic level, short-term transcriptomic responses between the locations diverged both in magnitude and metabolic pathways involved that correlate to a certain extent with local conditions (Chapter 4, Monteiro et al., 2019). Therefore, we suggest that population differentiation is already taking place and could be revealed at the physiological

and biochemical level by studies targeting extreme abiotic factor levels and/or longer exposure times.

Supplementary material

Table S1 Results of the one-way ANOVA or non-parametric test for effects of temperature on Fv/Fm during 7 days of temperature acclimation for *S. latissima* from Roscoff. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Source	df	MS	F ratio	p value
Fv/Fm day 4	Temperature	Kruskal-Wallis test			0.826
Fv/Fm day 7	Temperature	2	0.004	19.738	0.000*
	Error	12	0.000		

Table S2. Results of the two-way ANOVA or non-parametric tests for effects of temperature and salinity on Fv/Fm during 11 days of salinity acclimation for *S. latissima* from Roscoff. Statistically significant values are indicated by asterisks (p < 0.05).
The assumption of normality is not met for Fv/Fm on days 14 and 18. On day 11 the homogeneity of variances is marginally violated ($p = 0.045$).

Variable	Source	df	MS	F ratio	p value
Fv/Fm day 8	Temperature	Kruskal-Wallis Test			0.000*
	Salinity	Mann-Whitney U Test			0.161
Fv/Fm day 11	Temperature	2	0.013	27.897	0.000*
	Salinity	1	0.004	8.632	0.007*
	Temperature × Salinity	2	0.003	5.558	0.010*
	Error	24	0.000		
Fv/Fm day 14	Temperature	2	0.020	28.739	0.000*
	Salinity	1	0.000	0.197	0.661
	Temperature × Salinity	2	0.000	0.092	0.913
	Error	24	0.001		
Fv/Fm day 18	Temperature	Kruskal-Wallis Test			0.000*
	Salinity	Mann-Whitney U Test			0.217

Table S3. Results of the repeated measures ANOVA for effects of temperature and time on the fresh weight measured during 7 days of temperature acclimation for *S. latissima* from Roscoff. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Source	*df*	MS	*F* ratio	*p* value
Fresh weight	Within-subjects effects				
	Time	1	6520.131	50.088	0.000*
	Time × Temperature	2	686.803	5.276	0.018*
	Between-subjects effects				
	Temperature	2	2478.342	2.04	0.165
	Error	15	1214.693		

Table S4. Results of the repeated measures ANOVA for effects of temperature, salinity and time on the fresh weight measured during 11 days of salinity acclimation within three temperatures for *S. latissima* from Roscoff. Statistically significant values are indicated by asterisks ($p < 0.05$). The homogeneity of variance was violated for fresh weights on day 14.

Variable	Source	*df*	MS	*F* ratio	*p* value
Fresh weight	Within-subjects effects				
	Time	1.727	47899.896	56.069	0.000*
	Time × Temperature	3.455	3609.099	4.225	0.014*
	Time × Salinity	1.727	5160.091	6.040	0.011*
	Time × Temperature × Salinity	3.455	598.548	.701	0.651
	Error	20.730	854.303		
	Between-subjects effects				
	Temperature	2	32067.664	5.068	0.025*
	Salinity	1	40622.945	6.419	0.026*
	Temperature × Salinity	2	15668.730	2.476	0.126
	Error				

Table S5. Results of the two-way ANOVA or non-parametric tests for effects of temperature and salinity on pigments measured at day 8 for *S. latissima* from Roscoff. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*Df*	MS	*F* ratio	*p* value
Chl *a*[a]	Temperature	2	87609.966	6.015	0.008*
	Salinity	1	41711.920	2.864	0.104
	Temperature × Salinity	2	20440.669	1.403	0.265
	Error	24	14565.630		
VAZ	Temperature	2	0.002	0.831	0.448
	Salinity	1	0.004	1.509	0.231
	Temperature × Salinity	2	0.009	3.583	0.043*
	Error	24	0.002		
Acc[a]	Temperature	2	6736.289	6.186	0.007*
	Salinity	1	3426.996	3.147	0.089
	Temperature × Salinity	2	1284.950	1.180	0.325
	Error	24	1089.032		
DPS	Temperature	Kruskal-Wallis Test			0.006*
	Salinity	Mann-Whitney U Test			0.345

[a] after exponential transformation

Table S6. Results of the two-way ANOVA or non-parametric tests for effects of temperature and salinity on pigments measured on day 18 for *S. latissima* from Roscoff. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*Df*	MS	*F* ratio	*p* value
Chl *a*	Temperature	2	5.393	2.425	.110
	Salinity	1	0.018	0.008	.929
	Temperature × Salinity	2	0.282	.127	.882
	Error	22	2.224		
Acc.	Temperature	2	9.321	5.108	0.014*
	Salinity	1	0.083	0.046	0.833
	Temperature × Salinity	2	0.173	0.095	0.910
	Error	24	1.825		
VAZ	Temperature	2	0.012	1.128	0.340
	Salinity	1	0.000	0.030	0.865
	Temperature × Salinity	2	0.001	0.114	0.892
	Error	24	0.010		
DPS	Temperature	Kruskal-Wallis Test			0.002*
	Salinity	Mann-Whitney U Test			0.870

Table S7 Results of the two-way ANOVA or non-parametric tests for effects of time, temperature and salinity on mannitol (mg g^{-1} DW) measured on days 8, 11 and 18 for *S. latissima* from Roscoff. Effects of temperature and salinity were also tested independently by day. Statistically significant values are indicated by asterisks (*p* < 0.05).

Variable	Factor	*Df*	MS	*F* ratio	*p* value
Mannitol	Time	Kruskal-Wallis Test			0.155
	Temperature	Kruskal-Wallis Test			0.000*
	Salinity	Mann-Whitney U Test			0.010*
Mannitol_day8	Temperature	Kruskal-Wallis Test			0.001*
	Salinity	Mann-Whitney U Test			0.136
Mannitol_day11	Temperature	Kruskal-Wallis Test			0.001*
	Salinity	Mann-Whitney U Test			0.136
Mannitol_day18[a]	Temperature	2	1.138	217.744	0.000*
	Salinity	1	0.375	71.737	0.000*
	Temperature × Salinity	2	0.005	0.891	0.436
	Error	12	0.005		

[a] after log 10 transformation

Table S8 Results of the two-way ANOVA or non-parametric tests for effects of time, temperature and salinity on C:N, C (mg g^{-1} DW) and N (mg g^{-1} DW) measured on days 8, 11 and 18 for *S. latissima* from Roscoff. Effects of temperature and salinity were also tested independently by day. Statistically significant values are indicated by asterisks ($p < 0.05$). For N the assumption of normality is marginally violated ($p = 0.045$).

Variable	Factor	Df	MS	F ratio	p value
C:N	Time	Kruskal-Wallis Test			0.521
	Temperature	Kruskal-Wallis Test			0.000*
	Salinity	Mann-Whitney U Test			0.021*
C	Time	Kruskal-Wallis Test			0.094
	Temperature	Kruskal-Wallis Test			0.002*
	Salinity	Mann-Whitney U Test			0.000*
N	Time	2	63.333	17.303	0.000*
	Temperature	2	185.780	50.757	0.000*
	Salinity	1	1.168	.319	0.575
	Time x Temperature	4	21.112	5.768	0.001*
	Time x Salinity	2	9.952	2.719	0.076
	Temperature x Salinity	2	9.504	2.596	0.085
	Day x Temperature x Salinity	4	6.848	1.871	0.131
C:N_day 8[a]	Temperature	2	.040	100.220	0.000*
	Salinity	1	.004	9.519	0.006*
	Temperature × Salinity	2	1.254E-5	.031	0.969
	Error	18	.000		
C:N_day 11[a]	Temperature	2	.013	46.635	0.000*
	Salinity	1	.014	50.200	0.000*
	Temperature × Salinity	2	.003	10.061	0.001*
	Error	16	.000		
C:N_day 18	Temperature	Kruskal-Wallis Test			0.020*
	Salinity	Mann-Whitney U Test			0.203
C _day 8	Temperature	Kruskal-Wallis Test			0.001*
	Salinity	Mann-Whitney U Test			0.017*
C _day 11	Temperature	2	849.356	6.220	0.010*
	Salinity	1	2220.106	16.258	0.001*
	Temperature × Salinity	2	390.934	2.863	0.087
	Error	16	136.554		
C _day 18	Temperature	Kruskal-Wallis Test			0.691
	Salinity	Mann-Whitney U Test			0.283

[a] after log 10 transformation

Table S9 Results of the one-way ANOVA for the effect of geographical origin on F_v/F_m, fresh weight and pigment content measured on day 0 for *Saccharina latissima* from Roscoff and Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

						Sig.
Day 0_Area		Independent-Samples Mann-Whitney U Test				0.000*
Day 0_Photoprotection/ light harvesting		Independent-Samples Mann-Whitney U Test				0.008*
		Sum of Squares	df	Mean Square	F	Sig.
Day 0_ F_v/F_m	Between Groups	.005	1	.005	26.374	0.001*
	Within Groups	.002	8	.000		
	Total	.007	9			
Day 0_ VAZ/ Chl *a*	Between Groups	.003	1	.003	9.029	0.020*
	Within Groups	.002	7	.000		
	Total	.005	8			
Day 0_ Chl *a*	Between Groups	9.833	1	9.833	97.307	0.000*
	Within Groups	.606	6	.101		
	Total	10.439	7			

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6   Temperature modulates sex-biased gene expression in the gametophytes of the kelp *Saccharina latissima*

Temperature Modulates Sex-Biased Gene Expression in the Gametophytes of the Kelp *Saccharina latissima*

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Saccharina latissima is an economically and ecologically relevant kelp species in Europe and North America. In kelps, the sexuality is expressed during the haploid life stage and the microscopic gametophytes exhibit significant sexual dimorphism. To understand the sex-dependent impact of temperature on the gametophyte stage, we analyzed for the first time, gene expression profiles of male and female gametophytes at three different temperatures (4, 12, and 20°C) characteristic for the species distribution range by using RNA-sequencing. We identified several differentially expressed genes (DEGs) between sexes; while female biased genes were enriched in general metabolism and energy production, male biased genes function within cell cycle and signaling. In our study, temperature modulated sex-biased gene expression, with only a small percentage of DEGs consistently male (7%) or female-biased (12%) at the three temperatures. Female gametophytes responded stronger to higher temperatures than males, suggesting that males are more heat tolerant. Differences between *S. latissima* and other brown algal gender-dependent gene expression might mirror the different evolutionary and ecological contexts. Genomic information on kelp gametophyte is still scarce and thus this study adds to our knowledge on sex differences in abiotic stress responses in macroalgae at the transcriptomic level.

Keywords: brown algae, gametophyte, gene expression, kelp, life cycle, sex, temperature, transcriptomics

INTRODUCTION

Kelps (order Laminariales, Phaeophyceae) feature a haplo-diplontic life cycle in which microscopic haploid gametophytes alternate with macroscopic diploid sporophytes. The two stages are very distinct: sporophytes are structured in holdfast, stipe and blade featuring different tissues (cortex and medulla) and may reach up to several meters in length, while gametophytes are composed of

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one to few cells (Hurd et al., 2014b). These morphological differences between the life stages entail different susceptibility to abiotic stressors (Coelho et al., 2000), namely high irradiance, ultraviolet radiation, and temperature (e.g., Bolton and Lüning, 1982; Fredersdorf et al., 2009; Gao et al., 2019). Therefore, any prediction of future impacts of global warming on kelps must not only consider the responses of the sporophytes but also of the other life-history stages (Roleda, 2016; Assis et al., 2017; Leal et al., 2018).

Reproduction in kelps is tightly connected to environmental cues. Gametophytes may enter gametogenesis under the specific combination of nutrients, light, and temperature or remain vegetative if conditions are not favorable (Martins et al., 2017; Bartsch, 2018). In the latter case, vegetative gametophytes are considered to be dormant and may act as a "seed bank" allowing for the recovery of the population after a disturbance, e.g., storms or heat-waves (Edwards, 2000; Barradas et al., 2011). Moreover, vegetative gametophytes might be more resistant than macroscopic sporophytes to heat stress during summer, especially at the rear edge of the distribution. Lee and Brinkhuis (1986) suggested that the gametophyte and/or early life stages of Saccharina latissima "oversummer" at the southern distribution limit in United States, as sporophytes are not visible during the hot summer months but are already found in early autumn. Hsiao and Druehl (1973) reported gametophyte survival and gamete production of S. latissima throughout the year, however, absence of macroscopic sporophytes during later spring and summer. Given the challenges of studying the microscopic stages in the field, the relevance of the vegetative gametophyte pool for population dynamics is not well understood (Schiel and Foster, 2006). Nevertheless, manipulation of life-cycle transitions is a common practice in aquaculture to allow for year-round production of sporophytes (Charrier et al., 2017). Control of gametogenesis starts by separating male and female gametophytes based on their sexual dimorphism. After spore release and germination, sexual dimorphism soon becomes visible: while female gametophytes are constituted of a few bigger rounder cells that tend to grow in diameter, male cells are much smaller and increase faster in cell numbers (Lüning and Neushul, 1978). Under non-fertilizing conditions, male and female gametophytes form vegetative filaments consisting of several cells that are able to continuously grow over decades (e.g., Martins et al., 2019) and may form mm-sized clumps (Bartsch, 2018). The significance of sex to differences in performance, behavior, and ecology are also currently understudied in brown algae (Luthringer et al., 2014). Sexual dimorphism is to a large extent mediated by differential gene expression between sexes in a wide variety of organisms (Ellegren and Parsch, 2007). Up to the present, only a few gametophyte gene expression datasets are available for brown algae: Ectocarpus sp. (order Ectocarpales) (Lipinska et al., 2015), Undaria pinnatifida (Shan et al., 2015), Saccharina japonica (order Laminariales) (Ye et al., 2015), S. latissima (Pearson et al., 2019). Transcriptomic differences between sexes in Phaeophyceae have been seldomly explored, except for reproductive tissues in Fucus vesiculosus (Martins et al., 2013), S. japonica gametophytes (Bi and Zhou, 2014) Ectocarpus sp. gametophytes and gametes (Lipinska et al., 2013, 2015) and in

S. latissima gametophytes in response to gametogenesis induction and light quality changes (Pearson et al., 2019). Furthermore, so far, the influence of temperature has not been addressed. In the present study we provide the first data on transcriptomic responses of male and female gametophytes of the sugar kelp, S. latissima, to temperature. S. latissima is a relevant foundation species in temperate to Arctic coasts (Steneck et al., 2002; Teagle et al., 2017) and it is the selected species in several aquaculture facilities in Europe and North America (Forbord et al., 2012; Kim et al., 2015; Broch et al., 2018).

Our experiment targeted gametophytes of S. latissima originating from Helgoland (German Bight, North Sea) representing its center of distribution in respect to latitude (Araújo et al., 2016). Despite this, summer temperatures observed at this site are high (up to 20°C) (Breitbach et al., 2016) and close to the upper thermal tolerance limit of the species (Bolton and Lüning, 1982). Moreover, a decline in S. latissima relative presence and abundance has been described in Helgoland and were paralleled by increases in temperature and water clarity (Pehlke and Bartsch, 2008).

To our knowledge, this is the first study targeting brown algae gametophyte responses to temperature at the gene expression level. Hence, the present dataset will largely contribute to the understanding of the genomic basis of sex-specific differences in response to the environment of ecosystem engineering brown algae.

MATERIALS AND METHODS

Algal Material and Experimental Design

Gametophytes from uniparental stock cultures of Saccharina latissima [formerly Laminaria saccharina L. (Lamour)] (Lane et al., 2006) [AWI culture number 3094 (males) and 3096 (females)] from Helgoland, Germany were cultivated in glass flasks under red light (16hL:8hD) at 12°C (± 1°C) in an environmentally controlled room. Sterile seawater enriched with an adapted Provasoli medium without the addition of iron was replaced weekly. These conditions prevented the onset of gametogenesis (Lüning and Dring, 1975; Motomura and Sakai, 1981; Lewis et al., 2013), which was checked under the microscope regularly. To eliminate the confounding factor of red irradiance in our transcriptomic analysis, red light was replaced by white light with a photon fluence rate of 10–15 μmol photons m^{-2} s^{-1} (16hL:8hD) provided by fluorescent lamps OSRAM L 18W for 1 week prior to and during the experiment. These conditions retained our gametophytes' cultures at a vegetative stage. At the start of the experiment 3.10 ± 0.10 g of fresh weight of female gametophyte clumps and 2.20 ± 0.10g of male gametophytes clumps were each transferred separately to plastic petri dishes (diameter 9.5 cm × height 3 cm) (n = 4) and exposed to three temperatures (4, 12, and 20°C). Experimental temperatures were chosen to mirror mean minimum (4°C) and maximum temperatures (20°C) at Helgoland (Breitbach et al., 2016) and a control temperature (12°C) that falls within the optimal growth range for the species (Bolton and Lüning, 1982; Lee and Brinkhuis, 1988). Experiments were conducted in three

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environmentally controlled rooms and additionally temperatures were kept constant by circulating water baths (models Haake DC1, VWR 1136D). After 14 days of temperature exposure, gametophytes were sampled for RNA extraction. Samples were frozen in liquid nitrogen and stored at −80°C until further use. RNA extraction was performed within 3 weeks after sampling.

Maximum photosynthetic quantum yield of PS II (Fv/Fm) was measured at the start and end of the experiment ($n = 4$). Gametophytes were dark-adapted for 10 min prior to the measurements. Afterwards, Fv/Fm was measured with an Imaging PAM (Pulse Amplitude Fluorometer; Walz, Effeltrich, Germany).

Fresh weight ($n = 4$) was measured at the beginning and at the end of the experiment with a laboratory scale 40SM-200A (PRECISA, Switzerland). All data were tested for normality using the Shapiro–Wilk normality test and for homogeneity of variances using the Levene's test. Differences in growth and Fv/Fm were tested using a two-way ANOVA with the fixed factors temperature and sex. Significant differences and interaction of means were compared with the *post hoc* Tukey test (HSD). All statistical analyses were carried out using SPSS software version 25 (IBM, Armonk, United States). The significance level for all analyses was set at $\alpha = 0.05$.

RNA Extraction, Illumina Sequencing, and Data Processing

Total RNA extraction was conducted using the method described in Heinrich et al. (2012). Samples were first ground in liquid nitrogen. Then, samples were mixed with 1 ml extraction buffer (2% CTAB, 1 M NaCl, 100 mM Tris pH 8, 50 mM EDTA, pH 8) and 10 μL β-mercaptoethanol, followed by incubation at 45°C for 15 min. After, we added 1 mL chloroform. After mixing, samples were centrifuged for 20 min. 750 ml of supernatant were mixed with 1/3 volume of ethanol and 1 ml of chloroform. The tubes were then centrifuged again under the same settings as before. After centrifugation, RNA extraction was performed with a Qiagen Plant Mini Kit (Qiagen, Germany) following the manufacturer's protocol. RNA quality was analyzed by the NanoDrop ND-1000 UV-Vis Spectrophotometer and Agilent 2100 Bioanalyzer (Agilent Technologies, Germany). Three biological replicates per treatment were sequenced. cDNA libraries were prepared with an Illumina TruSeq RNA Library Prep Kit according to the manufacturer protocol. The libraries were sequenced on an Illumina Hiseq 2500 and 75 bp paired reads were clipped using default values of the Illumina software. Raw reads were quality controlled by FastQC[1] and quality filtered using Trimmomatic v. 0.36 (Bolger et al., 2014). Quality filtering was performed using the following parameters: leading 3, trailing 3, sliding window 4:15, minlen 30.

Reads from all treatments (three temperatures × two sexes) were assembled *de novo* with the RNAseq software Trinity v 2.4.0 with default parameters including the reads normalization step corresponding to the Trinity implementation of the diginorm method (Grabherr et al., 2011). The quality of the transcriptome assemblies was evaluated by using

BUSCO v3.0.2 (Waterhouse et al., 2017) applying an eukaryote dataset (OrthoDB v9.1). Gametophytes reads were pseudo-aligned with Salmon (Patro et al., 2017) against the *de novo* assembled transcriptome, the previously assembled sporophyte transcriptome (Li et al., 2019, Array express:E-MTAB-7348), the available *S. japonica* genome (Ye et al., 2015) and previously assembled transcriptomes of *S. latissima* (Heinrich et al., 2012; Jackson et al., 2017). A principal component analysis (PCA) plot of the counts-per-million, followed by a $\log_2$ transformation, of all treatments was generated by a Trinity script. Differential expression was calculated using DESeq2 (Love et al., 2014) at Trinity's gene level with an adjusted level of $p \leq 0.001$ and a $\log_2$ fold change of at least two indicating significance. Tools were executed using the scripts included in the Trinity package v 2.4.0 (Grabherr et al., 2011). Functional annotation was performed using the Trinotate functional annotation pipeline (Bryant et al., 2017) with the UniRef90 database as additional reference (all databases up to date in October 2017). In addition, annotation of transcripts via DIAMOND v0.9.13 similarity search (Buchfink et al., 2015), using "more-sensitive" option against the NR database (October 2017) was performed. Effects of temperature, sex and the interaction of temperature and sex were calculated using the multivariate analysis of variance function "adonis" of the package "vegan" (Dixon, 2003) in R studio version 1.1.442.

To investigate the function of significantly up- and down-regulated genes, Gene Ontology (GO) enrichments were conducted using GOseq (over-represented *p*-value < 0.05, Young et al., 2010). Results were summarized with CateGOrizer by applying GO slim (Hu et al., 2008). Venn diagrams were produced applying a webtool[2].

RESULTS

Photosynthetic Efficiency and Growth

To estimate overall fitness of the gametophytes, we assessed maximum photosynthetic quantum yield by measuring Fv/Fm and fresh weight at the beginning and end of the experiment. There was no significant effect of temperature and sex on fresh weight of gametophytes, measured as percentage of initial at the end of the 14 days exposure (temperature, $p = 0.159$, sex, $p = 0.525$). In all treatments a modest increase in fresh weight up to a maximum of 20% in females at 12°C was observed. No significant treatment changes were detected for Fv/Fm (data not shown).

De novo Transcriptome

To explore patterns of gene expression, we extracted RNA obtained from male and female gametophyte samples exposed to three different temperatures (4, 12, and 20°C). The number of reads per complementary DNA (cDNA) library ranged from 20 to 42 million with an average of 33 million reads. The remapping rate of gametophytes cDNA libraries on the *de novo* assembled transcriptome was on average 86% which is 5–18%

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[1] https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

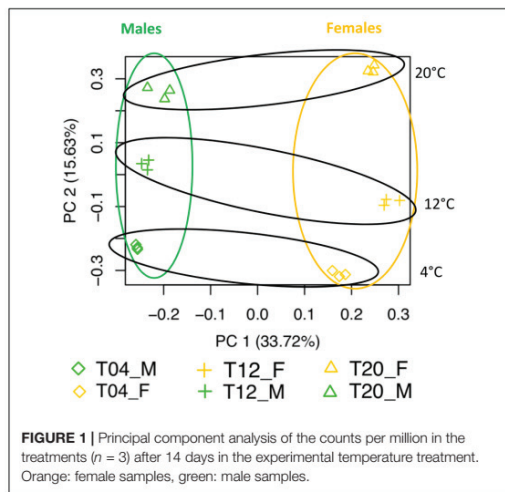[2] http://bioinformatics.psb.ugent.be/webtools/Venn/

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better than on other available transcriptomes assemblies (data not shown). The transcriptome assembly consisted of a total of 256,118 transcripts (corresponding to 211,947 Trinity genes) with an average contig size of 713 bp and N50 of 1350 bp[3] (**Supplementary Data Sheet 1 Table S1**). This high total number of genes is a common feature of the Trinity pipeline (e.g., Bryant et al., 2017). Ninety per cent of total expression was present in 58,741 of transcripts with a N50 of 1895 bp. Results of BUSCO analysis indicate that the assembled transcriptome has near-complete gene sequence information for 93% of the genes, 34% complete and single-copy BUSCOs, 59% completed and duplicated BUSCOs, 5% fragmented BUSCOs and 1% missing BUSCOs. 36,379 transcripts were functionally annotated using the UniProt Swiss-Prot database, 33,606 using Pfam and 60,961 with Uniref90 and 45,240 using NR. A total of 14,240 GO terms and 17,562 KEGG orthology (KO) were assigned to the transcripts (see text footnote 3; **Supplementary Data Sheet 1 Table S1**).

Sex-Biased Gene Expression at the Control Temperature

Several analyses performed in our dataset revealed distinct transcriptomic profiles between male and female gametophytes of *S. latissima*. Gender explained 34% of the variability in the transcriptome as shown in the PCA (**Figure 1**). The second axis of the PCA accounted for 16% of the variability and divided between the three temperatures tested. Gene expression profiles were significantly different between sexes ($p_{PERMANOVA}$ = 0.001) and between temperatures ($p_{PERMANOVA}$ = 0.009). Many genes were differentially expressed between males and females at the three temperatures (23,698 – 11.2%; **Figure 2**). Differential gene expression between sexes at the control temperature (12°C –

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[3]http://application.sb-roscoff.fr/blast/slatissima/

FIGURE 2 | Total number of significant differentially expressed genes (DEGs) (Deseq2, $p < 0.001$; $\log_2 FC > 2$, $n = 3$) in *Saccharina latissima* gametophytes. Up-regulated DEGs in male compared to female gametophytes are indicated in green, and orange in diagonal stripes bars, respectively.
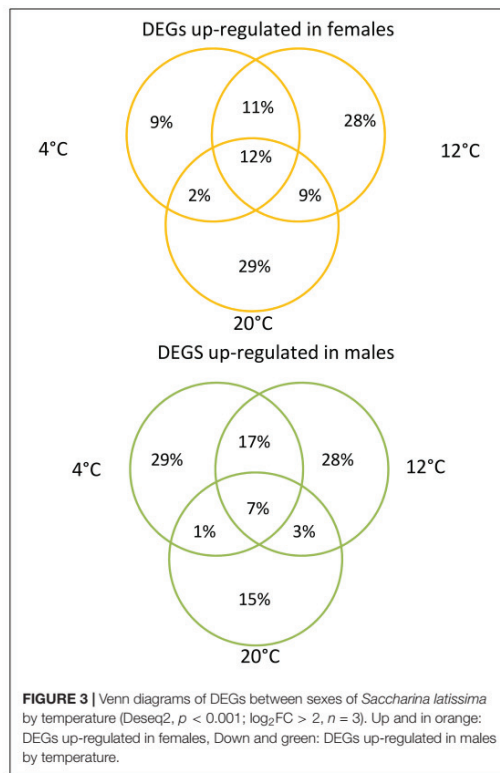
FIGURE 3 | Venn diagrams of DEGs between sexes of *Saccharina latissima* by temperature (Deseq2, $p < 0.001$; $\log_2 FC > 2$, $n = 3$). Up and in orange: DEGs up-regulated in females, Down and green: DEGs up-regulated in males by temperature.

FIGURE 1 | Principal component analysis of the counts per million in the treatments ($n = 3$) after 14 days in the experimental temperature treatment. Orange: female samples, green: male samples.

13,991 DEGs) was higher than at a temperature of 20°C (10,590) and at 4°C (9,411; **Figure 2**). Differential expression between sexes was more similar between 4 and 12°C than between 12

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and 20°C or 4 and 20°C (**Figure 3**). Across temperatures, a female-biased gene expression was observed, with more up-regulated DEGs in females compared to males. Among DEGs up-regulated in females, 2,072 (12%) were up-regulated at the three temperatures, 4,937 (29%) were uniquely up-regulated at 20°C, 4,798 (28%) at 12°C and 1,496 (9%) at 4°C. In
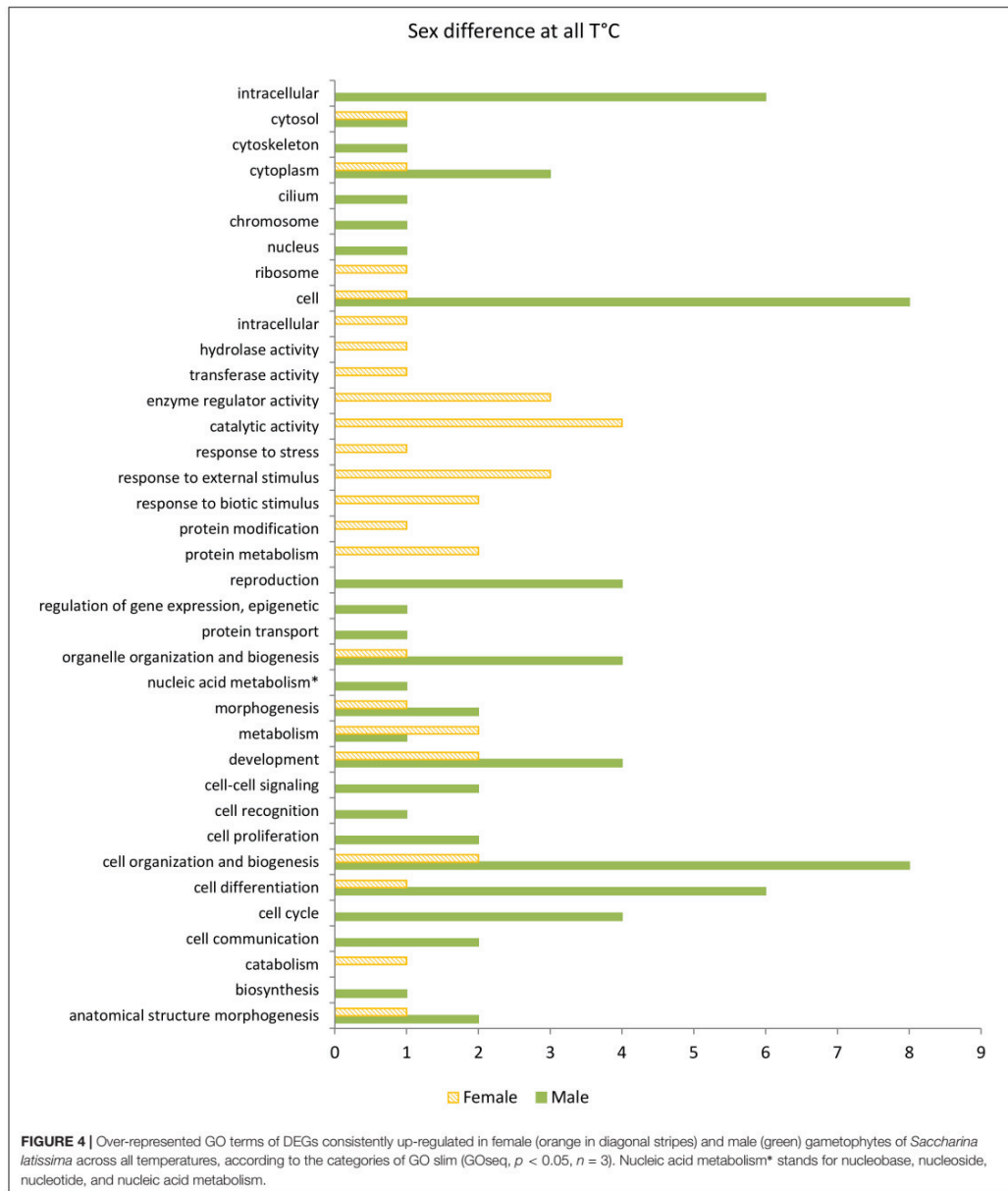
FIGURE 4 | Over-represented GO terms of DEGs consistently up-regulated in female (orange in diagonal stripes) and male (green) gametophytes of *Saccharina latissima* across all temperatures, according to the categories of GO slim (GOseq, $p < 0.05$, $n = 3$). Nucleic acid metabolism* stands for nucleobase, nucleoside, nucleotide, and nucleic acid metabolism.

FIGURE 5 | Over-represented GO terms belonging to the ontology Biological Process in male (green) and over-represented in female (orange in diagonal stripes) gametophytes of *Saccharina latissima* at 4, 12, and 20°C according to the categories of GO slim (GOseq, *p* < 0.05, *n* = 3). Nucleic acid metabolism* stands for nucleobase, nucleoside, nucleotide, and nucleic acid metabolism. Total for the category metabolism at 4°C – Females: 170, Males: 153, at 12°C: Females: 354, Males: 120, at 20°C: Females: 55, Males: 17.
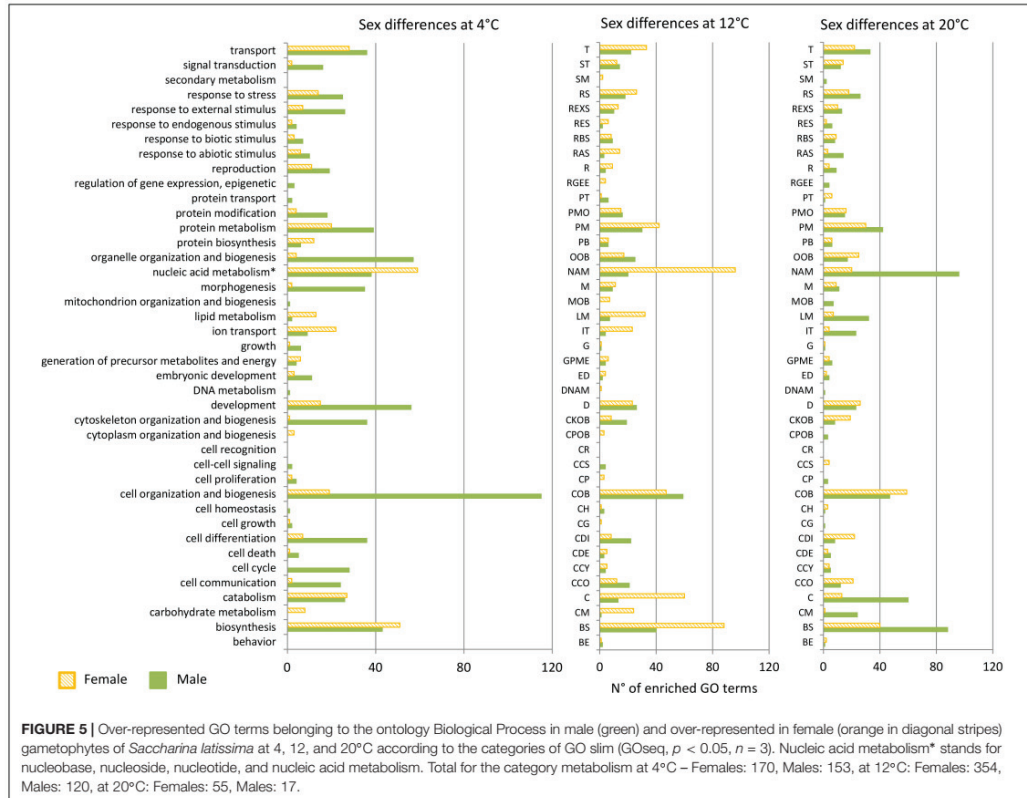
turn, among DEGs up-regulated in males, only 500 (7%) were consistent across temperatures, 1,930 (29%) were specifically regulated at 4°C, 1,873 (28%) at 12°C and 1,930 (29%) at 20°C (**Figure 3**). Consistently, several GO terms were significantly enriched in the comparison between gender and patterns shifted with temperature (**Figures 4**, **5**). The lower temperature, 4°C drove more metabolic reorganization in males than females and the opposite held true at 12 and 20°C for females. Taken together, these results reveal a clear sex-biased gene expression that was modulated by temperature.

Putative Sex Marker Genes

Analysis of the genes with the highest contribution to the Principal Component axis 1, measured by its loading values (Legendre and Legendre, 2012), revealed genes potentially related to sex determination in *S. latissima* (**Supplementary Data Sheet 1 Table S2**). Namely, one gene highly expressed in females and another one highly expressed in males both match one gene coding for homoaconitate hydratase that has been described as female-specific in *Ectocarpus* sp. and *S. japonica* (Lipinska et al., 2017). Also, a gene identified in six other brown algal species

as male-specific, the high mobility group protein 1 (Lipinska et al., 2017), was also male-specific in our data. Moreover, we identified two protein kinases: dual specificity protein kinase TTK and serine/threonine-protein kinase OSR1 associated with male gene expression. Two other genes functioning in protein modification, ubiquitin-conjugating enzyme E2 D2 and galactose-3-O-sulfotransferase 3 were associated with male samples. A gene coding for serine protease/ABC transporter B family protein tagC and a gene coding for calcium channel YVC1 were associated with females.

Functional Annotation of Sex-Biased Genes

To identify the functions of sex-biased genes, we performed a GO enrichment analysis in the set of genes consistently up-regulated in females (2,072) and males (500) across temperatures (**Figure 4**) and on the full set of genes differentially expressed between sexes at 4, 12, and 20°C (**Figure 5**). Sex-biased genes identified in this study were associated with several metabolic processes. Results of the GO enrichment analysis revealed that at 12 and 4°C the top 15 overrepresented GO terms were related to general metabolism, such as "peptide biosynthetic

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process," "organonitrogen compound biosynthetic process," and "translation" for both females and males up-regulated DEGs (**Supplementary Data Sheet 2 Table S3**). Terms related to cytoskeleton and signaling, e.g., "cellular component assembly," "actin filament organization," "small GTPase mediated signal transduction" were uniquely overrepresented in males while GO terms related to aminoacid metabolism ("carboxylic acid metabolic process") and nucleic acid metabolism ("nucleoside triphosphate metabolic process") were uniquely overrepresented in females at both 4 and 12°C. Considering the extensive list of enriched GO terms and that it contained several human-related biological processes, we applied GO slim to provide an overview. Among GO slim functional categories exclusively over-represented in females consistently across temperatures, we identified GO terms related to metabolism and responses to cues. In the category metabolism, the GO terms "protein metabolism" and "catabolism" were over-represented, while response to cues included the GO terms "response to stress," "response to external stimulus," and "response to biotic stimulus" (**Figure 4**). Accordingly, we identified considerably more stress response related DEGs up-regulated in females at the three different temperatures, namely heat-shock and antioxidant proteins (**Supplementary Data Sheet 1 Table S4**). In turn, functional categories solely over-represented in males included "cell-cell signaling," "cell cycle," and "cell proliferation." Moreover, some categories were over-represented in both males and females and could be connected to processes that we expect to take place in both sexes during gametophyte growth and development, namely "cell differentiation" and "cell organization and biogenesis."

Sex-Biased Gene Expression at Low and High Temperatures

Shifts in the expressed metabolic pathways were observed in response to changes in temperature. The low temperature tested, 4°C, drove more metabolic reorganization in males than in females (**Figure 5**). "Morphogenesis," "reproduction," "transport," and responses to endogenous, biotic and abiotic stimulus gathered more enriched GO terms in males than in females at 4°C (**Figure 5**), and the reverse was observed at 12°C. However, biological processes such as lipid and carbohydrate metabolism and "ion transport" were proportionally higher in females at 4°C and at 12°C than males. Concomitantly, "cell differentiation," cell and organelle organization and biogenesis were consistently over-represented in males compared to females at both 4 and 12°C.

At 20°C, we observed a decrease in the differentiation between sexes, revealed by the lower number of enriched GO terms within the root biological processes differentially expressed between males and females. Almost all the functional categories were over-represented in females: "transport," "signal transduction," "response to stress," "protein metabolism," "cell organization and biogenesis," and "cell communication" gathered the higher number of GO terms. The top 15 over-represented GO terms in females included "superoxide anion generation," "positive regulation of oxidative stress-induced

intrinsic apoptotic signaling pathway," "transposition," "DNA integration" (**Supplementary Data Sheet 2 Table S3**).

DISCUSSION

Sex-Biased Gene Expression Underlies Gender-Specific Metabolic Needs

In this study, we identified a high number of genes differentially expressed between male and female gametophytes which were in the vegetative stage. This pattern is consistent with descriptions for *Ectocarpus* sp. by Lipinska et al. (2015) who reported that differential gene expression between males and females was higher during the immature stage than during the fertile one. Furthermore, more female- than male-biased genes were identified, which agrees with results obtained during gametogenesis in *S. latissima* (Pearson et al., 2019). However, this is in contrast to the situation in two other brown algae, *F. vesiculosus* (order Fucales) (Martins et al., 2013), and *Ectocarpus* sp. (order Ectocarpales) (Lipinska et al., 2015). Although there are contrasting patterns in sex-biased gene expression within brown algae, the three species still belong to different orders and even high variability in sex-biased gene expression among species of the same genus has been described before (Metta et al., 2006). Moreover, such differences may mirror the different life cycles (Coelho et al., 2019). While Laminariales and Ectocarpales have a haplo-diplontic life cycle, Fucales display a diplontic life cycle. Moreover, the degree of sexual dimorphism in the haplo-phase (gametophytes) of Laminariales is stronger than in the order Ectocarpales and while the first produce anisogamous gametes with a large non-motile female egg cell and small motile male sperms, Ectocarpales gametes are both motile and isogamous (Luthringer et al., 2014).

Functional analysis of the differentially expressed genes (DEGs) revealed that several metabolic pathways are distinctly regulated between female and male gametophytes. In females, several GO terms related to general metabolism are enriched, such as "carbohydrate metabolism," "lipid metabolism," "nucleobase, nucleoside, nucleotide and acid metabolism." In addition, the over-represented GO term "generation of precursor metabolites and energy" suggest that female gametophyte cells are actively investing in cell growth and energy production. This is consistent with observational microscopy studies, as female gametophyte cells tend to grow in size while male gametophyte cells tend to grow in number (Lüning, 1980; Destombe and Oppliger, 2011; Park et al., 2017). This investment in cell growth might be advantageous for later sporophyte growth as larger female gametophyte cells will release bigger eggs that tend to generate larger sporophytes (Bell, 1997). Within lipid metabolism, we identified four genes coding for lipoxygenase that were sex-specific in *S. latissima* in this study (**Supplementary Data Sheet 1 Table S5**) and during gametogenesis (Pearson et al., 2019), as in *Ectocarpus* sp. (Lipinska et al., 2015) although female-specific in the first and male-specific in the latter species. Lipoxygenases are involved in lipid oxidation and play an important role in the biosynthesis of oxylipins – lipophilic mediators that have several physiological functions such as

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senescence, growth and development, tolerance to stress, and cell homeostasis (Hou et al., 2015; Maynard et al., 2018). In brown algae, they are involved in the defense response to pathogens and grazing (Cosse et al., 2009; Ritter et al., 2017) and their synthesis was induced under copper stress (Ritter et al., 2008). Considering the sex-biased gene expression of lipoxygenases in both *Ectocarpus* sp. and *S. latissima* we hypothesize that they are involved in regulating sexual differentiation in brown algae. Further work is required to understand the specific function of lipoxygenases in sexual differentiation and its different expression between brown algae species. In addition, two genes coding for mannuronan C5-epimerase were female-specific and one was male-specific in *S. latissima*, while in *Ectocarpus* sp. two genes were male and one was female-specific. Mannuronan C5-epimerases belong to large multigenic families that are involved in the synthesis of alginate, a major component of cell walls in brown algae (Michel et al., 2010). Previously, Fischl et al. (2016) reported four mannuronan C5-epimerases genes preferentially expressed in Ectocarpales gametophytes over sporophytes, suggesting a role in the gametophyte development. Differential expression of some of the genes encoding this enzyme might contribute to the different morphologies of the male and female gametophytes' cells.

A closer inspection of the functional annotation of DEGs revealed that some genes involved in oxidative stress responses were up-regulated in female gametophytes of *S. latissima*. Similar patterns were also reported for *S. latissima* during gametogenesis (Pearson et al., 2019) and in the female gametophyte of *Arabidopsis* (Wuest et al., 2010). Reactive oxygen species play a role in embryo sac development and fertilization in *Arabidopsis* (Martín et al., 2013), cell division (Livanos et al., 2012a) and cytoskeleton homeostasis (Livanos et al., 2012b) in plants. Thus, we hypothesize that reactive oxygen species might function as signaling molecules regulating gametophyte development, as previously reported in gender-biased gene expression in primates and mice (Yang et al., 2006; Blekhman et al., 2010), suggesting that these are highly conserved processes.

In vegetative males, GO terms in the GO slim functional categories "cell communication" and "cell-cell signaling" were over-represented which is probably connected to the fact that gametophyte maturation is dependent on both environmental factors and biotic cues, namely the release of pheromones (Lüning and Müller, 1978). Signal transduction might be crucial to allow for temporal and spatial overlap in gametophyte maturation which is critical for successful fertilization in kelps (Schiel and Foster, 2006). During our experiments no reproductive structures were visible and males did not receive female cues for maturation as sexes were kept separated. Nevertheless, our data suggests that male gametophytes prepare in advance for maturation at the transcriptomic level probably to be able to readily synchronize gamete release with females once specific cues are received. This might confer a competitive advantage and ensure successful fertilization. Li et al. (2013) have observed that the best fertilization rates in *S. japonica* occur 60–120 min after male gamete release and their viability is limited to 12 h. This is further supported by the expression of a "flagellar associated" gene reported from *Ectocarpus* which was only expressed in males of *S. latissima* at 4 and 12°C. Two "flagellar associated proteins" also displayed male-biased gene expression in immature gametophytes of *Ectocarpus* (Lipinska et al., 2015).

Furthermore, higher enriched GO terms within the categories "cell," "cytoskeleton," and "organelle organization and biogenesis" are in accordance with cell division being enhanced in male gametophyte filaments and these being more highly branched than female gametophyte filaments. Among these functional categories, we found two genes encoding DNA helicases with very high expression in males. DNA helicases are evolutionary conserved among eukaryotes and involved in DNA replication, namely by positive regulation of telomere length, replication of highly transcribed RNA polymerase II genes and unwinding G⁻quadruplex DNA structures (Rhodes and Lipps, 2015; Sabouri, 2017). Therefore, higher expression of these genes in males might be connected to higher cell division rates.

Changes in Temperature Modulated Sex-Biased Gene Expression

Functional analysis revealed that sex-biased gene expression shifted with temperature. Differences between sexes were less pronounced at 20°C than at 4 and 12°C suggesting that the gametophytes alter their metabolism from developmental and sex differentiation processes to a heat stress protection response that involves similar pathways in both sexes. This is in agreement with 4 and 12°C being more suitable temperatures for gametophyte growth and gametogenesis than 20°C (Lüning, 1980).

It is widely accepted that temperature is a major factor controlling reproduction in seaweeds (Egan et al., 1989; Andrews et al., 2014; Hurd et al., 2014a). This is further supported in our study by the interactive effect of temperature and sex in gene expression patterns in *S. latissima*. Only a small proportion of DEGs were consistent across temperatures (12% for females and 7% for males) and females responded stronger to the higher temperature (20°C) while more genes were overexpressed in males at lower temperatures (4°C). The effect of temperature on growth of *S. latissima* gametophytes has been previously demonstrated: Lüning (1980) observed optimum vegetative growth between 10 and 19°C and gametophyte death after 1 week at 22°C. Another study revealed that gametophytes survived 22°C and even 23°C but with visible cell damage (Bolton and Lüning, 1982). Hence, the higher temperature tested in the present study (20°C) is very close to the upper survival temperature in gametophytes and to the maximum temperature reached during summer at the original collection site of the gametophytes (Helgoland, North Sea, Breitbach et al., 2016). Other studies have suggested that male kelp gametophytes are slightly more thermotolerant than females (Lee and Brinkhuis, 1988; tom Dieck, 1993). This is supported by the differential gene expression of our study. The male gametophytes seem to be more resilient to the heat stress as functional categories such as "response to stress," response to external and abiotic stimuli and "cell death" were over-represented in females and categories such as "cell cycle" were over-represented in males. In addition, induction of transposable elements by stress and their effects on gene regulation has been observed in plants,

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namely in response to heat stress (Makarevitch et al., 2015; Dubin et al., 2018). The over-representation of GO terms related to transposition in females at 20°C suggests a role in heat stress response in *S. latissima* gametophytes. Concomitantly, other enriched GO terms connected to stress response, such as "superoxide anion generation," "positive regulation of oxidative stress-induced intrinsic apoptotic signaling pathway" were also among the top 15 over-represented GO terms in females at 20°C. This further supports the higher sensitivity to heat stress of female gametophytes. Assessing differential sensitivity to high temperatures between sexes in gametophytes is relevant for hybridization protocols aiming to produce more tolerant cultivars in aquaculture, as it is already current practice in crop plant species (Zinn et al., 2010). Recently, Martins et al. (2019) demonstrated that for the kelps *Laminaria digitata* and *L. pallida* female parent gametophytes contributed more for the thermal response of the hybrids than males. Our work suggests that the same might be true for *S. latissima* and informs current ongoing efforts to maximize sugar kelp cultivation practices.

Shifts in sex ratio in response to sub-optimal temperatures have already been described in several kelp species (Izquierdo et al., 2002; Nelson, 2005; Oppliger et al., 2011). In *S. latissima*, Lee and Brinkhuis (1988) described a greater proportion of male compared to female gametophytes at higher temperatures (between 17 and 20°C). Female gametophyte grew at 20°C, but with a lower rate than between 4 and 17°C and their fecundity was repressed. Pronounced temperature-mediated sex ratio shifts might lead to population declines in several species, especially at sites where species are close to their upper thermal tolerance limit, and may have an impact on the range distribution of species (Janzen, 1994; Ospina-Alvarez and Piferrer, 2008; Hays Graeme et al., 2017). Differences in the sex ratio between populations along the distributional range have been observed for several plants (Barrett and Hough, 2012) and for the kelp *Lessonia nigrescens* (Oppliger et al., 2011), which warrants concern that skewed sex ratios due to global warming might compromise populations viability. At the gametophyte stage, global warming may lead to a mismatch between male and female gametophyte performance that might compromise sexual maturation and fertilization, as has been recently highlighted by de Bettignies et al. (2018). At the same time, the high levels of gene expression reported here indicate that vegetative gametophytes are still metabolically active rather than "dormant" as in plant seeds (Schiel and Foster, 2006) and therefore more vulnerable to abiotic stress. Therefore, the sex differences in gene expression patterns in response to temperature described here for *S. latissima* prompts for further investigation on the vulnerability of the gametophyte life-stage to temperature and its potential effects in reproductive success and phenology.

AUTHOR'S NOTE

This manuscript has been released as a Pre-Print at https://www.biorxiv.org/content/10.1101/750455v1.

DATA AVAILABILITY STATEMENT

The Illumina sequence reads generated during the present study are available in the Array express repository under the accession number E-MTAB-8267 (Athar et al., 2019; Monteiro et al., 2019) (https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-8267/). The transcriptome assembly, peptide and annotation generated in this study are available on http://application.sb-roscoff.fr/blast/slatissima/index.html.

AUTHOR CONTRIBUTIONS

CM performed the experiment, carried out the analyses and RNA extractions, analyzed the data, and wrote the manuscript. EC assembled the *de novo* transcriptome and performed the bioinformatics analyses related to RNA – sequencing data with help from LH. KB, IB, SH, JC, EC, LH, and KV supervised the project and helped with the data interpretation and discussion. CM, SH, KB, IB, and KV designed the experimental set-up. IB further provided the algae cultures. GG carried out the RNA-sequencing and commented on the manuscript. All authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2019.00769/full#supplementary-material

TABLE S1 | Summary of transcriptome statistics. Summary of transcript annotations in the public databases.

TABLE S2 | Top 100 genes contributing to PC1 of the Principal Component analysis according to their loading values.

TABLE S3 | Top 15 over-represented GO terms by sex (male, female) and temperature (4°C, 12°C, and 20°C), both total GO terms and sex-exclusive.

TABLE S4 | List of stress response related DEGs between sexes at the three different temperatures.

TABLE S5 | List of DEGS between sexes found in common in *Ectocarpus* sp. and *S. latissima*.

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7 Synoptic discussion

7.1 Kelp transcriptomics under temperature stress

7.1.1 Transcriptomics for revealing stress responses in plants, seaweeds and kelp

Transcriptomics is a widely used tool to assess responses of organisms to environmental change. Signalling pathways inform the cell of new conditions that require and trigger extensive regulation at both transcriptional and post-transcriptional level. Hence, gene expression profiles reveal which metabolic pathways are induced and/or repressed following an environmental cue. Furthermore, key genes in stress response can be identified (López-Maury et al., 2008; Evans, 2015). A set of genes is commonly induced under stress and is conserved across distantly related organisms. This set referred to as "cellular stress response" is involved in several processes such as protein chaperoning and repair, DNA repair, redox regulation, cell cycle control, lipid and energy metabolism (Kültz, 2005). This cellular stress response is common to several stress factors (thus also designated as core stress response), however some components are stressor- and species- specific (Collén et al., 2007; Evans and Hofmann, 2012; Holzinger et al., 2014). Molecular chaperones are involved in several processes enabling protein homeostasis, such as transport, correct folding and unfolding and assembly of aggregated proteins. Among chaperones, heat-shock proteins (HSPs) are well-known examples of genes expressed under stress conditions across taxa and are therefore considered stress biomarkers (Sørensen et al., 2003). Another component of this response is redox regulation (Hurd et al., 2014b). Several stressors induce the production of reactive oxygen species (ROS) in the cell. In photosynthetic organisms, ROS are the byproduct of photorespiration, photosynthesis and mitochondrial respiration. Accumulation of ROS may lead to lipid peroxidation, protein oxidation, DNA damage and eventually to cell death. Thus to counteract these effects, ROS-scavenging enzymes scavenge peroxide and reduce the levels of ROS in the cells and antioxidants such as glutathione and ascorbic acid are produced. Common ROS-scavenging enzymes in plants include superoxide dismutase, ascorbate peroxidase and catalase. Caution must be taken when correlating induction of redox regulation processes with environmental stress, as ROS are important messengers in signaling and are therefore involved in other biological processes (Mittler, 2002). Moreover, response to stress and cellular differentiation share signaling pathways in Eukaryota (Wuest et al., 2010). Therefore, induction of cellular stress response in female over male gametophytes (Chapter 6) might be connected to differentiation processes rather than to response to temperature or might function in both processes. Similarly, other metabolic pathways are induced and/or repressed in response to more than one environmental or internal cue and therefore dissecting the exact role under specific stress conditions is challenging. While some genes are induced in response to stress, others are commonly repressed. Repressed genes often function within translation, ribosome and protein synthesis that ultimately have an impact on growth. Above a species-specific stress threshold, energy will be completely diverted to a stress response and growth will be impaired (López-Maury et al., 2008).

Furthermore, modulation of gene expression is dependent on the severity of applied stress. While a moderate stress level induces transcriptional reprogramming that nevertheless allows the organism to survive and even maintain their physiological performance, severe stress levels will lead to proteolysis and eventually cell death.

Several studies have confirmed that stress biomarkers, such as HSPs and antioxidant enzymes are also modulated by stress in seaweeds as in other taxa, e.g. the red alga *Chondrus crispus* (Collén et al., 2007), Fucales *Fucus vesiculosus* (Mota et al., 2018), *F. serratus* (Jueterbock et al., 2016) and kelps *Saccharina japonica* (Liu et al., 2014), *S. latissima* (Heinrich et al., 2015) *Laminaria digitata* (Roeder et al., 2005). Part of this work was carried out by measuring only certain HSPs genes by quantitative polymerase chain reaction (qPCR) (Jueterbock et al., 2014; Mota et al., 2018). This targeted analysis has advantages compared to genome-wide analysis (e.g. reduced costs). However, several lines of evidence suggest that other pathways beyond the cellular stress response are often also triggered by stress (Sørensen and Loeschcke, 2007; Hwang et al., 2008). Thus, the approach taken in this thesis – to study global expression by RNA-sequencing – provides a wide picture of metabolic pathways at play in response to stress that might be overlooked with other approaches.

Transcriptomic analysis targeting *S. latissima* is currently limited to the work of Heinrich and colleagues (2012) and this thesis. While Heinrich et al. explored interactive effects of temperature, irradiance and/or UV, the present thesis targets interactive effects of temperature vs. salinity and temperature vs. sex. These studies have in common the response to the temperature factor as one of the most relevant environmental drivers influencing fitness, survival and reproduction, and ultimately geographic distribution (Breeman, 1988; Parmesan and Yohe, 2003; Bartsch et al., 2008; Andrews et al., 2014). Comparison between the present and former studies is challenging considering that two different methodologies were applied: RNA-sequencing here and microarrays in the former. This might result in a higher annotation rate in the data originating from microarrays as the number of total transcripts is usually lower (Wang et al., 2009). Moreover, experimental temperatures applied differed between Heinrich et al and this thesis and between sporophytes (Chapter 4) and gametophytes (Chapter 6). Sporophytes from the Arctic and Brittany were exposed to ecologically relevant temperatures in either one or the other location (0 °C, 8 °C and 15 °C; Chapters 3, 4 and 5). Experimental temperatures applied to male and female gametophytes were chosen to mirror the temperature range experienced at the site of origin: Helgoland, German Bight, North Sea (4 °C, 12 °C and 20 °C; Chapter 6). This precludes a direct comparison of response to temperature between life stages. Nevertheless, it provides valuable ecological information on the gametophytes of the Helgoland population that can also be applied to more southern populations as summer temperatures are similar. Still, we attempt to summarize common patterns of response to temperature across these experiments by using KOG categories (Figure 8). The Clusters of Orthologous Groups of proteins (COGs) database with its extension Eukaryotic Orthologous Groups (KOG) is a common tool used for gene annotation (Tatusov et al., 2003). The KOGs assigned to proteins are further classified into 25 functional categories independent of each other (Figure 8). These categories provide an overview of expressed pathways and have been used to compare gene expression profiles across different experimental set-ups and even species (e.g. Strader et al., 2016).

KOG categories with the highest percentage of expressed genes across all treatments were [J] Translation, ribosomal structure and biogenesis, [K] Transcription, [L] Replication, recombination and repair, [T] Signal transduction mechanisms, [O] Posttranslational modification, protein turnover, chaperones, [G] Carbohydrate transport and metabolism and [I] Lipid transport and metabolism. This pattern reveals that the algal response to temperature involves a wide range of pathways. In turn, KOG categories with very little expression were [V] Defense mechanisms, [N] Cell motility, [W] Extracellular structures, [Q] Secondary metabolites biosynthesis, transport and catabolism (Figure 8). In addition, temperature varia-

tion promoted expression of genes functioning within a wider array of KOG categories in gametophytes than in sporophytes. Both up and down regulation in gametophytes covered genes expressed in almost all KOG categories, which reveal that although gametophytes are anatomically simpler than sporophytes, they display a complex set of cellular processes. Moreover, the number of expressed genes in response to temperature was higher in gametophytes than in sporophytes (Table 2). This is in accordance with a recent comparison between sporophyte and gametophyte expression profiles in two species of Laminariales that revealed that gametophyte-biased expressed genes were surprisingly relatively high even though a correlation was found between the extent to which life stages differ in size and complexity and the number of life stage biased expressed genes (Lipinska et al., 2019). Furthermore, the model brown alga *Ectocarpus*, with sporophyte and gametophyte morphologically similar, expresses a higher number of genes at the gametophyte than at the sporophyte stage (Arun et al., 2019). Overall, an increase in temperature leads to stronger changes in gene expression than a decrease in temperature (Figure 8). An exception can be noted for sporophytes for Spitsbergen at 0 °C and 2 °C, where several categories belonging to metabolism were expressed. In addition, when comparing patterns of expression in sporophytes of Roscoff and Spitsbergen at 0 °C, categories such as lipid transport and metabolism, ion transport and metabolism and coenzyme transport and metabolism are only up-regulated in Spitsbergen, suggesting that *S. latissima* in the Arctic is able to maintain metabolic activities and use, for example, energy from lipids to maintain fitness at lower temperatures. At the gametophyte level, as already described in Chapter 6, female gametophytes responded in a stronger fashion to high temperatures than male gametophytes, both in number of DEGs and in number of cellular processes involved (Table 2 and Figure 8). This differential response between sexes to temperature has already been reported in kelps (Lee and Brinkhuis, 1988; tom Dieck, 1993; Liu et al., 2016), but remains poorly explored. Global warming induced changes in reproduction through namely shifts in sex-ratios and compromised reproductive success has been considered one of the most relevant impacts compromising species performance and survival (Ospina-Alvarez and Piferrer, 2008; Hedhly et al., 2009; Zinn et al., 2010; Johnstone et al., 2017).
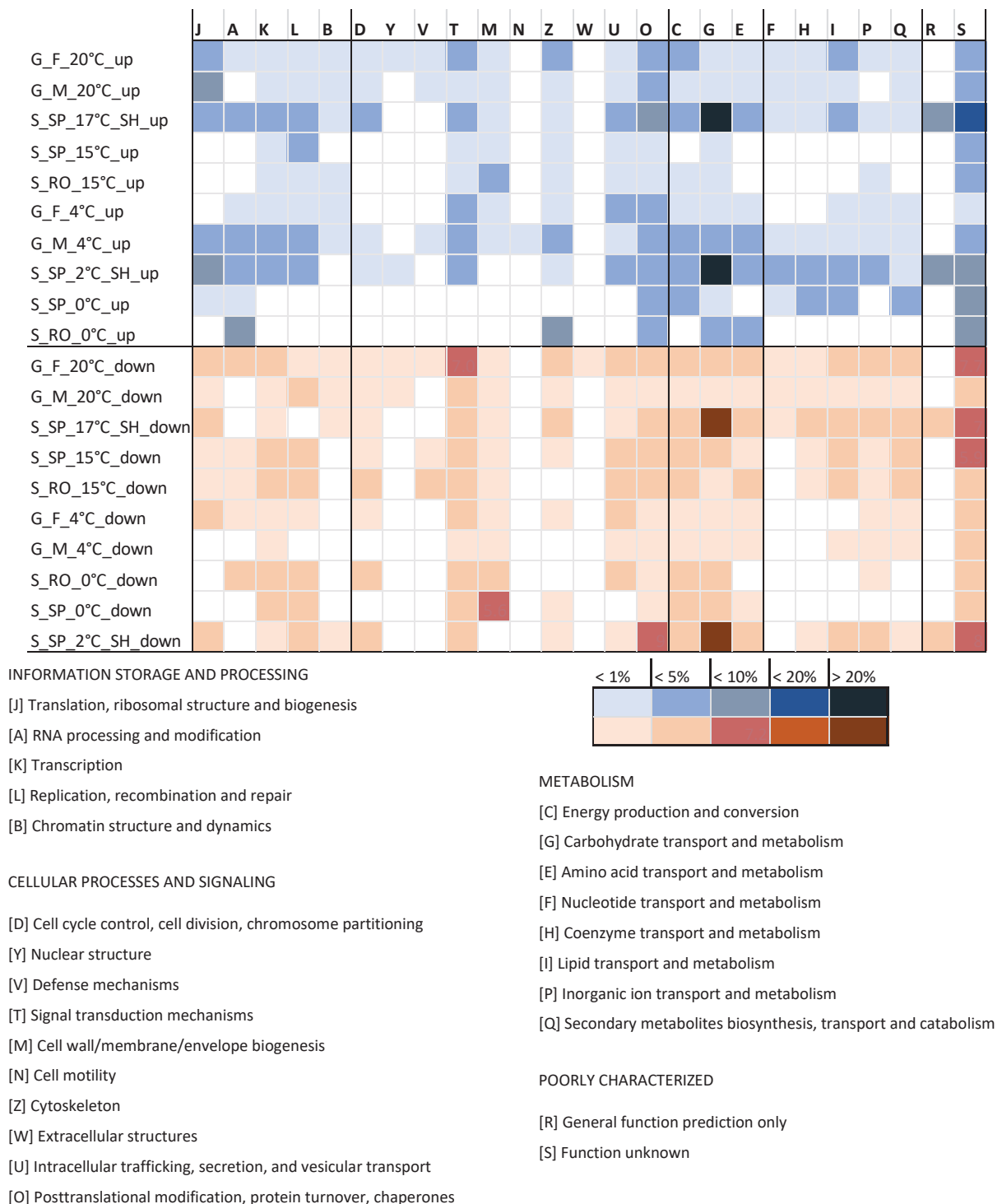
Figure 8 KOG category distributions of differentially expressed genes in response to temperature. The figure displays the KOG category distribution of up (blue, upper part of the graph) and down (red, lower part of the graph) in comparison to the control treatment of each experiment. Control temperature was 8 °C for the experiments with sporophyte (S), 12 °C for the gameto-phytes (G) and for Heinrich et al.'s data (SH). Color intensities reflect the amount of genes per group calculated in percent of total DEGs grouped into KOGs with known or general function prediction. S: sporophyte, G: gametophyte, SP: Spitsbergen, RO: Roscoff, SH: Heinrich et al.'s data (Heinrich et al., 2012b), F: female, M: male

Table 2 Number of DEGs regulated (DEseq2, p < 0.001; log2FC > 2) in comparison to the control temperature of each experiment. Control temperature was 8 °C for the experiments with sporophytes (S) and 12 °C for the gametophytes (G). S: sporophyte, G: gametophyte, SP: Spitsbergen, RO: Roscoff, F: female, M: male

	up-regulated DEGs	down-regulated DEGs
G_F_20 °C	2377	2095
G_M_20 °C	1107	836
G_F_4 °C	406	667
G_M_4 °C	1163	592
S_SP_15 °C	211	230
S_RO_15 °C	319	1063
S_SP_0 °C	130	166
S_RO_0 °C	37	133

7.1.2 Is there evidence for local adaptation in kelps?

Genetic analysis of *S. latissima* throughout the distributional range revealed significant genetic differentiation between populations. This degree of genetic differentiation is suggested to underlie a complex of incipient species (= a subspecies in the process of becoming permanent and thus changing to a true species) in Europe (Guzinski et al., 2016; Neiva et al., 2018). Likewise, McDevit and Saunders (2010) identified three mitotypes in *S. latissima* – Pacific, European and (Northwestern) Atlantic – that are consistent with an incipient species complex hypothesis. Moreover, observations of low genetic diversity within populations and isolation between them limit the breadth of responses available for the species in terms of phenotypic plasticity and adaptation. Aligned with the molecular evidence, several studies reported ecotypes based on physiological and biochemical measurements. Müller et al. (2008) identified differences in germination between zoospores originating in Helgoland and Spitsbergen: germination was inhibited at 18 °C in Spitsbergen while remaining at 80% for the Helgoland samples. Moreover, hybridization experiments between gametophytes of the two populations resulted in significantly lower sporophyte production than crossings within same population. Ecotypic differentiation between Spitsbergen and Helgoland populations is further supported by findings by Olischläger et al. (2014) that uncovered significant differences in biochemical composition at 10 °C and a differential response to CO_2 between sporophytes from the two populations. In a later study, differences between growth and photosynthetic performance were also revealed for the same populations (Olischläger et al., 2017). In contrast, Bolton and Lüning (1982) detected no population specific response to temperature (0 °C, 5 °C, 10 °C, 15 °C, 20 °C and 23 °C) between *S. latissima* populations of Helgoland (Germany), Brest (France), Isle of Man (United Kingdom) and Espegrend (Norway) in cultured sporophytes and gametophytes. The contrasting gene expression profiles reported in this thesis between sporophytes from Roscoff (Brittany, center of distribution) and Spitsbergen (Arctic, northern limit of distribution) further suggest the existence of ecotypes in *S. latissima* (Chapter 4). Although it is not possible to draw definitive conclusions about local adaptation from our experiment, this work is the first to contribute to the current body of information with transcriptomic data. Our results suggest that responses to temperature and salinity variation have diverged between sporophytes from Roscoff and Spitsbergen. This is supported by the findings A) gene expression profiles of sporophytes from Roscoff and Spitsbergen were clearly distinct (as revealed by

the principal component analysis), B) several differentially expressed genes were identified between algae from the two locations at the control conditions and C) transcriptomic reprogramming was to a certain extent performed differently by algae from Brittany and the Arctic.

As observed at the transcriptomic level, physiological measurements also diverged between sporophytes from the two locations. For all physiological measurements obtained (e.g. Fv/Fm, pigments and growth) there was a significant difference between algae from Roscoff and Spitsbergen at the start of the experiment (Chapter 5). Growth (measured as percentage of initial) was higher in Spitsbergen sporophytes than Roscoff ones at the end of the experiment. However, initial fresh weight was higher in Roscoff specimens than Spitsbergen. During cultivation, algae have been growing under the same temperature and irradiance conditions (8 °C, 20 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR)) but in higher density therefore with fewer nutrients and less light available than during the experiment. We would expect that the Spitsbergen population would perform better under low light as they are exposed to less irradiance in the Arctic than Roscoff; therefore it is surprising that at first sporophytes from Roscoff grew faster and then slowed down growth compared to Spitsbergen. We do not expect the nutrients to be a major factor here as the Provasoli solution added in full (20ml for 1L) is well above natural seawater available nutrient levels. Is it possible that given that Spitsbergen sporophytes are usually exposed to sub-optimal conditions with high seasonal restrictions, they react faster to optimal conditions in order to maximize growth. In the case of local adaptation, we would expect Spitsbergen's sporophytes to grow better at 0 °C than Roscoff ones but the opposite should hold true at 15 °C (reflecting the environment of origin), which was not the case. It is also puzzling that Roscoff sporophytes grew slightly better at 8 °C than 15 °C. However, this difference was not significant, and only a significant decrease in growth rate at 0 °C was observed when compared to 8 °C and 15 °C. In conclusion, ecotypic differentiation between algae from Roscoff and Spitsbergen in growth could not be confirmed by this experiment.

Parallel with the differential expression analysis, we identified several constitutively expressed genes under the control condition of our experiment (Chapters 3 and 4). Differential expression analysis might fail to detect significant fold changes if genes are already expressed at high levels under the control treatment (meaning constitutively expressed genes). Constitutively expression of stress response related genes, such as HSPs and ROS might underlie phenotypic plasticity in response to stress and therefore lead to the wide acclimation potential of the species. However, mRNA production comes with an energetic cost – several ATP molecules are required during production of pre-mRNA and for the processing from pre-mRNA to mature RNA. Some studies have shown that increases in gene expression leads to a reduced fitness (Dekel and Alon, 2005; Lang et al., 2009). Namely, a trade-off between growth rate and sexual reproduction was observed in the yeast *Saccharomyces cerevisiae*. Sterile individuals had higher growth rates and this was the result of down-regulation of the mating signaling pathway genes (Lang et al., 2009). Dekel and Alon (2005) compared the cost (protein expression) to the benefit (higher growth rate) of the utilization of lactose by *Escherichia coli* based on the *lac* system. They reported that benefit of lactose is decreased in high lactose environments due to the high cost of protein expression. Moreover, they reported a rapid evolutionary adaptation towards an optimal cost-benefit scenario (Dekel and Alon, 2005). On the contrary, Chan et al. (2012) reported a lack of correlation between the magnitude of

transcriptomic changes and the effects in phenotype and fitness in two of the three transgenic plants of *Arabidopsis thaliana* manipulated to increase tolerance to salt stress. Plants overexpressing the gene *CBF3* suffered a significant reduction in growth, reproductive development, leaf number, rosette diameter, plant height, dry weight and seed yield. These extensive impacts on fitness and reproduction have already been reported in other studies (Liu et al., 1998; Achard et al., 2008), where increased tolerance to freezing was associated with retarded growth. However, transgenic plants overexpressing *M6PR* and *SOS1* exhibited little effects on phenotype. Differences can be explained by the fact that the three genes modulate salt tolerance through different mechanisms. Higher impact on fitness in plants over-expressing *CBF3* is probably due to the fact that this gene is a transcription factor hence its expression affects a wider range of metabolic pathways (Gilmour et al., 2004). Chan et al. (2012) also reported differences in response between their study that targeted long-term response and previous results focusing on short-term effects. This study highlights the intricate relationship between gene expression and fitness and that it is gene-specific. Tani et al. (2019) compared the responses of two species of shrub of the same genus with different tolerance to drought stress. Higher tolerance to drought of *Medicago arborea* was associated with higher expression of antioxidant related genes and slower growth when compared to the less tolerant *M. alborea* and their hybrid.

Therefore, also for *S. latissima* it is likely that a trade-off arises between a broader tolerance based on costly constitutive gene expression versus a local-driven response to environmental fluctuation that reduces transcriptomic costs but narrows the tolerance range (Latta et al., 2012). Moreover, from an early stage of cultivation the sporophytes developed clear distinct morphologies (Chapter 4). Algae from Roscoff were wider and shorter while algae from Spitsbergen presented long but narrow lamina. At the transcriptomic level, several genes related to cell wall and membrane adjustment were identified to be differentially expressed between sporophytes from Roscoff and Spitsbergen at the control conditions which might underlie the differences in morphology. Differences in morphology across the distributional range of seaweeds is common and often reflect environmental variation (Serisawa et al., 2002; Matson and Edwards, 2006; Rothman et al., 2017). In the brown alga *Fucus guiryi* morphologies vary across a northern to southern gradient accompanied with an increase in temperature (Monteiro et al., 2017). In *F. vesiculosus* inhabiting the Baltic sea morphology is a function of salinity and exposure (Kalvas and Kautsky, 1993). Here, distinct morphologies between populations were evident although genetic differentiation was missing, suggesting that morphology is a plastic trait (Rinne et al., 2018). In *S. latissima*, the effect of wave exposure in morphology has also been described both in field (Chapman, 1974; Peteiro and Freire, 2013) and laboratory observations (Gerard, 1987). Spurkland and Iken (2012) described morphological variation between *S. latissima* populations from an oceanic and a glacially-influenced site in Canada. Sporophytes from the glacially influenced site were narrower and longer than the oceanic sporophytes. This morphology is in accordance with the morphology described here for the glacially influenced site (Spitsbergen, Arctic). Reciprocal transplants and *in situ* growth measurements in Canada suggest that these differences are genetically fixed (Spurkland and Iken, 2012), as it has been described in other brown algae (e.g. Stengel and Dring, 1997), and similarities with our study indicate that this trait is maintained even across large geographical scales.

Variation in seaweed morphology affects nutrient uptake (Wallentinus, 1984) photosynthetic capacity (Stewart and Carpenter, 2003; Miller et al., 2006), sensitivity to mechanical stress (Buck and Buchholz, 2005; Koehl et al., 2008), among others, and are thus likely to significantly impact species performance

(Stengel and Dring, 1997; Hurd et al., 2014d). Although morphology variation in response to water movement has been extensively described, the relationship between morphology and temperature is less well understood (Gerard, 1987; Hurd, 2000). Nevertheless, we can expect that *S. latissima* exposed to different temperatures will display different photosynthetic rates and nutrient requirements (Davison, 1991; Machalek et al., 1996; Gerard, 1998) and hence morphological variation might compensate for this (Kübler and Dudgeon, 1996). Thus further research targeting modulation of morphology along latitudinal clines should elucidate which factors regulate morphology and the relevance of this trait in the response to environmental variation (Gao et al., 2016). Furthermore, correlating commercially desirable morphological traits with location would inform aquaculture site selection (Peteiro and Freire, 2013).

Taken together, hints of local adaptation in our experiment might indicate that, given enough time, *S. latissima* might fully adapt to the site environmental conditions and will then reduce stress responses and be able to redistribute energy expenditure to processes such as growth and reproduction. While local adaptation allows species to thrive in the different sites it might limit species response to new, unexpected stressors. Therefore, the apparent acclimation potential of *S. latissima* within a framework of ongoing local adaptation might allow for the resilience of the species under current environmental conditions. However, it is already abundantly evident that this resilience has limits that have been crossed in several locations and led to decreases in *S. latissima* occurrence and abundance (e.g. Eriksson et al., 2002; Pehlke and Bartsch, 2008; Casado-Amezúa et al., 2019). Future research should include temperature levels higher than the ones tested here and closer to the upper thermal tolerance of the species as this might provide a better picture of possible adaptation to the local conditions.

7.1.3 Does transcriptomics match physiology?

Previous work suggested that *S. latissima* might experience sub-optimal environmental conditions in the Arctic as its temperature optimum for growth lies between 10 and 15 °C (Fortes and Lüning, 1980; Bolton and Lüning, 1982). Our work supports this statement as the higher temperature tested in our experiments with sporophytes revealed that 15 °C promoted physiological performance, namely growth, accessory pigments content and maximum quantum yield (Fv/Fm) of photosystem II (Chapter 3). The gene expression results also reveal that 15 °C was not particularly stressful. 15°C promoted more differentially expressed genes than 0 °C, however the number of up-regulated genes were similar (130 for 0 °C and 211 for 15 °C) since a high proportion of DEGs regulated at 15 °C were down-regulated. Functional analysis supported by GO enrichment revealed that up-regulated DEGs at 15°C functioning mostly within the classes "Metabolism", "Signaling", "Transport" and "Stress response" represented only 9%. Moreover, genes belonging to the cellular stress response (discussed above) were very seldom induced at 15 °C. Therefore, the species might benefit from warming in the Arctic and this might pose a competitive advantage over the endemic kelp *Laminaria solidungula* (Iñiguez et al., 2016; Scheschonk et al., 2019). Similar results were obtained for the algae from Roscoff (Chapter 5).

However, increased ice melting due to increasing temperatures in the Arctic has other consequences that might counteract the apparent beneficial effect of temperature. Namely, increasing ice melt will result in increased turbidity and therefore degradation of the light climate and increased sedimentation

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which has already been shown to negatively impact *S. latissima* (Spurkland and Iken, 2011; Bartsch et al., 2016; Zacher et al., 2016). Moreover, increased freshwater input and precipitation events will reduce the salinity experienced by the species. Hyposalinity effects in *S. latissima* have also already been described (Karsten, 2007; Spurkland and Iken, 2011; Nielsen et al., 2016; Mortensen, 2017) and were revealed in our study at both physiological and transcriptomic level (Chapters 3, 4 and 5).

After 24 hours of exposure to low salinity, extensive reprogramming took place at the transcriptomic level. On the contrary, effects of hyposalinity on F_v/F_m values, a measure of algal fitness, were more subtle (nonetheless, significant at certain time points) and only the xanthophyll-cycle pool, among the pigments measured, was affected in algae from both Roscoff and Spitsbergen (Chapters 3 and 5). Nevertheless, growth rate was significantly reduced at low salinity after 11 days of treatment in algae from both locations. Although no data on gene expression are available for this time point, we can hypothesize that effects at the physiological level are revealed later than at the transcriptomic level. An alternative hypothesis is that algae were able to acclimate to short-term hyposalinity (one day) by metabolic reorganization that ensured no negative effects in the physiological parameters. However, when exposed for longer periods (eleven days), the algae are no longer able to withstand the effects of low salinity.

An ameliorating effect of high temperature was observed at the transcriptomic level for algae from both locations (Chapters 3 and 4). The treatment 15 °C and low salinity drove less DEGs than low salinity at 0 °C and 8 °C. In contrast, interactive effects at the photo-physiological level were less prominent. In algae from Roscoff higher VAZ on day 8 and lower F_v/F_m on day 11 at 0 °C low salinity was observed but not at higher temperatures. Accessory pigments on day 11 were lower at 0 °C than at higher temperatures only at low salinity in the Arctic algae. However, overall the extensive repression of photosynthesis related DEGs was not mirrored in the pigments and F_v/F_m measured. Similarly, even though we detected a stronger response of female compared to male gametophytes to 20 °C, no significant response was observed on F_v/F_m and fresh weight (Chapter 6). Other studies have already reported significant changes at the transcriptomic level without a significant change in physiology in seaweeds (Heinrich et al., 2015; Iñiguez et al., 2017). Hence, gene expression is a more sensitive parameter than several physiological parameters measured to date. However, studies combining these approaches are still limited, and more research integrating different levels of organization (e.g. proteomics, metabolomics) is needed to understand how the responses are modulated from the gene to the physiology level (Dittami et al., 2011; Ritter et al., 2014; Liu et al., 2019).

Interactive effects at the gene expression level in *S. latissima* have been described previously. In cultivated sporophytes, 2 °C and 7 °C promoted the double of differential expressed genes than 12 °C in response to UVR after 24 hours (Heinrich et al., 2016) and similarly, after two weeks exposure under the same conditions (Heinrich et al., 2015). At the functional level, a stronger oxidative stress response to UVR happened at the lower temperature 2 °C compared to 12 °C. In addition, interactive effects between temperature and light levels were also revealed for *S. latissima*, with high temperature (17 °C) and high PAR representing the treatment with the largest impact at the transcriptional level (Heinrich et al., 2012b). The previous studies highlight the relevance of studying the interaction of abiotic factors since the outcome can substantially diverge from single factor experiments. Temperature seemed to have an ameliorating effect until a certain level, but very high temperatures resulted in stress related

responses. Similarly, we described the interaction of temperature and salinity effects in this study and, to the best of our knowledge, for the first time at the transcriptomic level in kelps (Chapters 3 and 4). We observed that transcriptomics and physiology are not always clearly related. Hence, the choice of parameter to measure depends on the goal of the study and should be considered carefully. As investment in RNA production is energetically costly for the cell, gene expression is a relevant indicator to detect an early-response that might lead to reduced fitness.

7.2 Sex-specific differences in kelp transcriptomics

Brown algae (Phaeophyceae) display a complexity of life cycles with variable degree of phenotypic differentiation, reproductive systems and different levels of sexual dimorphism. Therefore they constitute an interesting group to study from an ecological and evolutionary perspective (Coelho et al., 2019). The life cycle of algae within the order of Laminariales includes a macroscopic life stage, the sporophyte, and several microscopic stages: spores, gametophytes and gametes (see Figure 2 in Introduction). Sex is expressed at the haploid stage (gametophyte and gamete) that presents sexual dimorphism. Sexual dimorphism is displayed across the tree of life. The same genome has to express either male or female phenotypic traits and therefore sexual dimorphism is to a large extent dependent on differential gene expression between sexes. A large proportion of the genome has been described to be differentially expressed between males and females (Ellegren and Parsch, 2007). The degree of sexual dimorphism has been shown to be connected to the extent of gene expression – taxa with more evident sexual dimorphism show a larger transcriptomic reprogramming (Jiang and Machado, 2009; Pointer et al., 2013; Lipinska et al., 2015a). In brown algae, it has also been show that higher morphological distinction between sporophytes and gametophytes is broadly associated with higher life stage-specific gene expression (Lipinska et al., 2019). In Chapter 6, we describe evident sex-biased gene expression in *S. latissima* with a higher proportion of differentially expressed genes up-regulated in females. This can be connected with morphological and physiological differences observed in male and female gametophytes of *S. latissima*. Female gametophyte cells tend to be larger than male gametophyte cells and male gametophyte filaments are constituted of more cells than female ones. This sexual dimorphism can be associated in our dataset with an enrichment of general metabolism (carbohydrate and nucleic acid metabolism, generation of energy) in female gametophytes and with an enrichment of GO terms related to cell division and signaling in male samples. Moreover, we identified several genes that are sex-specific (only expressed in one of the sexes) and/or sex-biased (expressed in higher proportion in one of the sexes) in *S. latissima*. These genes represent potential sex markers that have several applications, such as breeding programs in aquaculture, population and demography research and evolutionary studies of sexual reproduction and therefore warrant further investigation (Lipinska et al., 2015b; Zhang et al., 2018). In addition, we studied the effect of temperature on sex-biased transcriptome profiles of gametophytes of *S. latissima*. Sex-specific transcriptomic responses to abiotic stress have, to the best of our knowledge, not been studied in seaweeds so far. However, evidence from land plants indicates that resilience to abiotic stress can vary significantly between males and females and this is also revealed at the gene expression level (Zinn et al., 2010; Jiang et al., 2012; Sage et al., 2015). Temperature is one of the main factors determining reproductive success in kelps (Lüning, 1990; Demes and Graham, 2011; Liu et al.,

2017) as in several other organisms, such as plants (Zinn et al., 2010). Namely, temperature was the main factor driving spore germination, female gametophyte growth and female fecundity in *S. latissima* (Lee and Brinkhuis, 1988). In addition, seasonality seems to play a role in gametophyte growth and maturation. Lee and Brinkhuis (1986) reported that the time of the year the meiospores were produced subsequently influenced the optimal temperature and light conditions for the survival, growth and reproduction of gametophytes. Although female gametophyte growth was possible at 20 °C, the growth rate depended on the season the spores were collected: growth rate at 20 °C was higher in summer than winter, which reveals a seasonal adaptation to temperature that ensures year-round gamete production. In addition, a shift from sexual to asexual reproduction in stressful environments was observed in several plants and algae, namely a tendency for asexual reproduction at the species geographical margins (Peck et al., 1998; Eckert, 2002). Therefore, the susceptibility of reproduction to temperature is cause for concern under the current and future warming scenarios (Hedhly et al., 2009). Considering that several environmental conditions (nutrients, temperature, light quality and light intensity) play a role in determining gametogenesis success and gametophyte growth and that these processes seem to follow a seasonal pattern that might change between established ecotypes, further research that includes more populations, more abiotic factor levels and the interactive effects of those is needed to complement current knowledge.

7.3 Conclusions

The aim of this thesis was to explore acclimation to abiotic stress in the sugar kelp *Saccharina latissima* under current environmental and future scenarios resulting from global warming. *S. latissima* currently experiences highly variable environments along its distributional range and several acclimation mechanisms have already been described. The study contributes to the current body of knowledge of physiological and biochemical responses to the abiotic factors temperature and salinity. In addition, the transcriptomic data contributes to the understanding of the genomic basis of acclimation in brown algae that has so far been mostly limited to the well-studied *Ectocarpus sp*. and *Saccharina japonica*. Conclusively, the research Questions put forward in Chapter 1.8. can be answered as follows:

1. Does acclimation to temperature in sporophytes of *S. latissima* increases tolerance towards salinity stress?

Interactive effects of temperature and salinity on gene expression profiles were identified for sporophytes of *S. latissima* after 24 hours (Chapters 3 and 4). Low salinity treatments promoted more transcriptional changes than temperature alone. Higher temperatures reduced the effect of hyposalinity. However, a temperature acclimation before the low salinity treatment did not seem to reduce the negative effect of salinity variation. At the physiological level, interactive effects were less evident. Overall, higher temperatures promoted growth, pigment content, and maximal quantum yield of photosystem II (F_v/F_m). In turn, hyposalinity decreased growth and F_v/F_m. In addition, mannitol content increased in response to low temperature and decreased in response to low salinity in sporophytes from Roscoff. Therefore, we predict that hyposalinity events expected to be more frequent and extreme in the future due to global warming will affect *S. latissima* performance. This would be most relevant to the hydrographic conditions in Arctic fjord systems or coastlines characterized by semi-enclosed embayments with limited water exchange. Further studies should include more severe salinity variation and its interactions with other abiotic factors as this will inform predictions of range shifts in *S. latissima*.

2. Do sporophytes of the populations from the North (Arctic) and Center (Brittany) distributional range present the same transcriptomics and/ or physiological responses to stress?

Differences between the transcriptomic profiles of sporophytes originating in the central (Roscoff, Northeast Atlantic) and northern distribution (Spitsbergen, Arctic) of the sugar kelp *Saccharina latissima* were revealed (Chapter 2). In a common garden laboratory experiment, sporophytes raised from gametophytes under similar controlled temperature and salinity conditions responded differently to changes in these abiotic factors that reflect the environmental conditions of the original sites. Specifically, Roscoff sporophytes exhibited a stronger response to low temperature and low salinity at the transcriptomic level, while in sporophytes from Spitsbergen higher temperatures exerted a stronger effect.

At the physiological level, growth rates of sporophytes from Spitsbergen and Roscoff did not reflect an enhanced fitness at conditions of the site of origin. Nevertheless, some physiological responses diverged between sporophytes of the two locations and morphological differentiation was evident from an early stage of cultivation. Hence, we cannot undoubtedly conclude that there is ecotypic differentiation be-

tween Roscoff and Spitsbergen. Nonetheless, considering the extent to which both transcriptomics and physiological parameters diverge, we suggest that ecotypes could be revealed with further experiments. Physiological performance of *S. latissima* sporophytes originated in the Arctic was enhanced at 15 °C revealing that the species might benefit from increasing temperatures predicted for the region. However, lower salinities had the opposite effect suggesting that this abiotic factor might compromise the success of the species in a warming Arctic (Chapter 3).

3. Is there a sex-biased gene expression in the vegetative gametophytes of *S. latissima*?

Transcriptomic profiles differ between sexes and underlie differences in morphology, physiology and ecology (Chapter 6). A female-biased gene expression was observed. Genes up-regulated in females generally function within general metabolism and energy production while genes up-regulated in males participate in cell cycle and signaling. This reveals an effort into cell growth in females in contrast with an effort in cell division and signal perception in male gametophytes. In addition, we identified sex-biased genes that can be applied in sex determination to facilitate breeding in seaweed cultivation and in evolutionary and ecologically studies in seaweeds as species-specific sex-biased gene expression was identified.

4. Is there an interaction between temperature and sex effects in vegetative gametophytes of *S. latissima* at the transcriptomic level?

Temperature modulated sex-dependent gene expression responses in gametophytes of *S. latissima*. Proportion of female-biased genes increased with increasing temperature. Higher temperatures (20 °C) drove more metabolic reorganization in females, including stress related pathways, revealing that female gametophytes are more heat sensitive than males (Chapter 6). The differential heat tolerance between sexes has a potential effect in reproductive success at the rear edge of distribution and will be increasingly relevant under the current warming trends. Thus, this finding has strong implications on species reproductive success in the face of global warming and on breeding programs for aquaculture.

In conclusion, transcriptomics can be used as an early warning indicator of emerging stress before adverse effects on physiology are detected. However, it should be considered that interpretation of results is more intricate than the interpretation of physiological measurements. Moreover, molecular mechanisms driving acclimation in *S. latissima* are many fold and go beyond known cellular stress responses, with likely high energetic costs. *S. latissima* sensitivity to hyposalinity is likely to impact performance at the leading edge and locally throughout the distributional range where freshwater input and precipitation events are likely to increase, such as in the Baltic sea. Furthermore, sex-dependent sensitivity to high temperatures in gametophytes of *S. latissima* might compromise sexual reproduction at the rear edge of distribution and in sites where the species is close to the upper thermal tolerance limit. These results have implications in predictions of range shifts in the species distribution.

7.4 Future perspectives

The applications of the transcriptomic data generated during this thesis do not expire here. As sequencing costs continue to drop and research interest continues to rise, more genomic resources of brown algae can expected to be available in the near future. These resources, namely the genome of *S. latissima*, will broaden the applications of the current data. Transcriptomic data can be integrated with relevant proteomics, metabolomics and genomics data in system biology approaches that will provide a view of the complex interplay of the different systems (Dittami et al., 2011; Ritter et al., 2014; Liu et al., 2019). Furthermore, modulation of gene expression by epigenetics (= "all mechanisms that potentially regulate gene expression, such as DNA methylation, histone modifications and variants, and noncoding and antisense RNAs.", Torda et al., 2017) has recently been demonstrated in brown algae, namely histone modifications (Bourdareau, 2018) and DNA methylation (Fan et al., 2019). The coupling of RNA-sequencing with techniques that detect histone modifications (e.g. chromatin immunoprecipitation (ChIP)) pave the way for a better understanding of regulation of gene expression, namely in life-history transitions (Bourdareau, 2018; Fan et al., 2019) and potentially in response to stress (Maumus et al., 2011). Moreover, gene expression information gathered here can be further applied to evolutionary studies, namely the rate of evolutionary divergence between sex-biased and non-biased genes, as performed in *Ectocarpus sp.* (Lipinska et al., 2015a). On the other hand, gene expression data can be integrated with phenotypic traits through approaches such as weighted gene co-expression network analysis (WGCNA) (Massa et al., 2013; Kenkel and Matz, 2016; Morandin et al., 2016). In addition, detecting Single Nucleotide Polymorphisms (SNPs) from expressed genes offers advantages over genomic data and can be applied in population genomics (De Wit et al., 2012; Jueterbock et al., 2016).

To uncover phase-specific responses to environmental factors further studies shall include direct comparisons between life-history stages. As stated before, microscopic life-history stages are still considerably understudied compared to the sporophyte stage (Bartsch et al., 2008). Further studies should include spores, other phases of the gametophyte development, gametes and microscopic sporophytes. A complete picture of sensitivity/resilience across the life cycle will reveal the weak link in kelps' resilience to ongoing anthropogenic threats.

To better understand geographical gradients in *S. latissima* investigations throughout the distributional range of the species will contribute to distinguish between contributions of phenotypic plasticity and adaptation underlying the biogeography of the species and its possible modulation by climate change scenarios. Studies including temporal patterns will reveal the critical time-point in species survival and uncover the relevance of seasonality in shaping species response to environmental changes.

List of abbreviations

ANOVA	Analysis of variance
APX	L-ascorbate peroxidase
C:N	Carbon to nitrogen ratio
CAT	Catalase
Chl	Chlorophyll
DEGs	Differentially expressed genes
DNA	Deoxyribonucleic acid
DW	Dry weight
FAD2	Delta-fatty-acid desaturase
FAD7	Sn-2 acyl-lipid omega-3 desaturase
Fv/Fm	Maximal quantum yield of photosystem II
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GO	Gene ontology
GSTs	Glutathione S-transferases
HPLC	High-performance liquid chromatography
HSPs	Heat shock proteins
IMTA	Integrated multi-trophic aquaculture
MsrA	Peptide methionine sulfoxide reductase
N	Nitrogen
mRNA	Messenger Ribonucleic Acid
NTRC	Thioredoxin reductaseNTRC
PAR	Photosynthetic active radiation
PAR	Photosynthetically active radiation
PCA	Principal component analysis
PES	Provasoli enriched seawater
PS II	Photosystem II
qPCR	quantitative polymerase chain reaction
RNA	Ribonucleic acid
ROS	Reactive oxygen species
S_A	Absolute salinity
SD	Standard deviation
SOD	Superoxide dismutase
TPM	Transcripts per kilobase million
VAZ	Xanthophyll cycle pigments
V-BPOs	Vanadium dependent bromoperoxidases

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