Effects of multiple abiotic stresses on gene expression in *Saccharina latissima* (Phaeophyceae)

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Effects of multiple abiotic stresses on gene expression in *Saccharina latissima* (Phaeophyceae)

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Erklärung gemäß § 6 (5) der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche vom 14. März 2007

Hiermit erkläre ich, Sandra Heinrich, dass ich die Arbeit mit dem Titel

„**Effects of multiple abiotic stresses on gene expression in *Saccharina latissima* (Phaeophyceae)"**

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SUMMARY

Marine macroalgae are globally distributed on rocky coastal shores, from tropical to polar regions. They are important marine coastal primary producers, and of particular importance to the function of many ecosystems. Kelps, brown algae of the order *Laminariales*, dominate rocky shores of cold-temperate regions. There, they help to structure the biodiversity of coastal ecosystems by forming huge forests, which provide habitats and nurseries for various marine organisms. The distribution of kelps is constrained by abiotic factors like light including UV radiation and temperature. Future global environmental changes could therefore have a potentially significant impact on geographic distribution patterns, vertical zonation, and primary productivity of kelp. The basic physiological and ecophysiological characteristics of kelps are well studied. Several physiological studies have been performed on kelp, primarily on the effects of single abiotic stressors, e.g. UV radiation and temperature. Only a few projects have focused on the interactive effects of multiple stresses. So far, no study is available on the molecular processes underlying physiological acclimation to abiotic stress factors in these important organisms. This thesis represents the first large-scale transcriptomic study of acclimation to abiotic stress in a kelp species, and aims on investigating molecular mechanisms underlying physiological acclimation to multiple abiotic stresses in *Saccharina latissima* from the Arctic (Spitsbergen).

Young sporophytes of *Saccharina latissima* were exposed in multifactorial experiments to different combinations of photosynthetically active radiation, UV radiation and temperature for durations of 8h, 24h and 2 weeks. In order to observe the degree of photoinhibition in response to different exposure conditions, maximum quantum yield of PS II (Fv/Fm) was measured at the beginning and at the end of the experiments. A cDNA library from RNA sampled under various light and temperature regimes was constructed for subsequent functional genomic studies on the mechanisms and pathways involved in stress acclimation to multiple stressors. Gene expression profiles under abiotic stress were assessed by microarray hybridizations. Thereby two different stress exposure durations, 24hours and 2 weeks, were applied to distinguish molecular mechanisms of short-term versus long-term acclimation to stress. Finally, a comparative approach investigating gene expression profiles in both laboratory and field grown sporophytes was carried out to elucidate interactive effects of UVR, temperature and growth conditions.

The established cDNA library consists of 400,503 ESTs, which were assembled to 28,803 contigs. Sequence comparison by BLASTx, Interpro protein-motif annotation, and Gene Ontology (GO) yielded in putative functions or orthology relationships for over 10,000 contigs. Comparative analysis with the genome of *E. siliculosus* revealed high functional genomic coverage of 70% of the cDNA library. The cDNA library is representative of the *S. latissima* transcriptome under the tested conditions and displays a rather complete gene catalogue of the species. It therefore constitutes an excellent basis for subsequent functional genomic studies on molecular acclimation to multiple stresses in *Saccharina latissima.*

S. latissima responds to abiotic stress with a multitude of transcriptional changes. Temperature had a smaller influence on metabolic processes than light. Two main temperature effects were observed. On the one hand, induction of genes associated with the glycine, serine and threonine metabolism in response to low temperature, and on the other hand repression of transcripts encoding carbohydrate biosynthetic and catabolic processes at high temperature. General stress responses observed in sporophytes subjected to high PAR include induction of catabolic processes for energy supply, heat shock proteins and antioxidant enzymes.

The combination of the stress factors high PAR, UVR and temperature caused interactive effects on photosynthesis and gene expression. Thereby excessive light at 17°C was the most destructive stress condition for *S. latissima*, resulting in a strong repression of several crucial metabolic processes, e.g. photosynthesis, carbohydrate metabolism and amino acid metabolism. Acclimation to high irradiance at low temperatures includes enhanced induction of glycine, serine and threonine metabolism, potentially as a consequence of a higher demand of glutathione (GSH), a reducing co-factor for several enzymes involved in reactive oxygen species (ROS) detoxification.

Reactive oxygen species formation (ROS) displays a central element of abiotic stress response. The observed regulation of various ROS scavenging enzymes in response to temperature, high PAR and UVR stress demonstrates the crucial role of ROS metabolism in acclimation to abiotic stress in *S. latissma*. Interestingly, gene expression data bear evidence for the existence of compartment specific ROS scavenging mechanisms in *S. latissma.* Furthermore, sophisticated regulation of Hsps was observed, which is involved in acclimation not only to temperature but also to combined environmental stresses such as high PAR in combination with high temperature.

Short- and long-term acclimation to UVR includes enhanced regulation of photosynthetic components, e.g. light harvesting complex proteins, thylakoid proteins and proteins associated with both photosystems. Gene expression analysis showed that photosystem II exhibits a higher susceptibility towards UV radiation than photosystem I. Furthermore, repair of UV damaged PS II reaction centre seems to function by increasing the transcript pool for transcripts associated with PS II. The observed induction of vitamin B_6 biosynthesis after all short- and long-term UVR treatments seems to be a crucial component of UVR acclimation in *Saccharin latissima*. Only short acclimation to UVR caused enhanced regulation of DNA replication and DNA repair. Three different DNA repair processes, photoreactivation, homologous recombination, and nucleotide excision repair were detected, indicating a sophisticated regulation of different DNA repair processes. As no enhanced regulation of DNA metabolism was detected after the 2 weeks UVR exposure experiments, *S. latissima* seems to be able to acclimate to UVR radiation and to overcome the negative effects of UV radiation on DNA.

Finally, comparisons of gene expression profiles in field and cultivated sporophytes were conducted. Large differences in gene expression between cultured and field material were observed. Principal effects of UVR, targeting mostly photosynthesis and DNA, were similar in cultured and field sporophytes, demonstrating laboratory experiments being well suited to investigate basic molecular mechanisms of acclimation to abiotic stresses. The study revealed that field sporophytes exhibit a higher susceptibility to UVR and a higher oxidative stress level at 12°C, whereas cultivated sporophytes in contrast must make stronger efforts to acclimate to UVR at 2°C. These findings are mostly due to the different growth temperatures of between -3°C and 1°C for field sporophytes versus 10°C for cultivated sporophytes. However, the results indicate that cold acclimation of *S. latissima* from the field caused metabolic alterations to increase stress performance at low temperatures, which concurrently led to higher susceptibility at 12°C.

This thesis presents an initial idea on the complexity of molecular acclimation to abiotic stress in *Saccharina latissima*. The molecular data obtained by this study improve our understanding on stress response in a kelp species and provide a useful platform for future research regarding molecular approaches in kelp. This project furthermore demonstrates the importance of research on interactions of abiotic stresses on both the physiological as well as on the molecular level. The results of the comparative gene expression study in cultured and

field sporophytes highlight the influence of growth conditions on molecular acclimation to stress and underscore the importance of conducting experiments with field material, when aiming to predict effects of changing abiotic factors in the field.

ZUSAMMENFASSUNG

Marine Makroalgen sind von tropischen bis polaren Regionen weltweit verbreitet und stellen wichtige Primärproduzenten und Schlüsselorganismen in Küstenökosystemen dar. Braunalgen der Ordnung Laminariales (Kelps) dominieren die Felsküsten kalt-gemäßigter Regionen, wo sie große Unterwasserwälder bilden, die als Habitat, Nahrungsgrundlage und Kinderstube für viele verschiedene marine Organismen dienen und so ein wichtiges Strukturelement in Küstenökosystemen darstellen. Die geographische Verbreitung, aber auch das vertikale Zonierungsmuster der Laminariales wird durch abiotische Faktoren, wie z.B. Licht und Temperatur, bestimmt. Daher ist anzunehmen, dass die globalen Umweltveränderungen die Verbreitung, Tiefenverteilung und Leistungsfähigkeit der einzelnen Arten verändern.

Die grundlegenden physiologischen und ökophysiologischen Eigenschaften der Kelp-Arten sind gut erforscht. In vielfältigen physiologischen Studien wurden bisher hauptsächlich Untersuchungen zu den Auswirkungen einzelner abiotischer Faktoren, wie z.B. UV-Strahlung und Temperatur, durchgeführt. Die Reaktion von Kelp auf interaktive Einflüsse aus dem Zusammenwirken mehrerer Faktoren hingegen ist bisher weitgehend unerforscht. Trotz ihrer großen ökologischen Bedeutung sind die auf molekularer Ebene zugrunde liegenden Prozesses der physiologischen Anpassung an abiotische Stressoren bei Kelps nur lückenhaft untersucht. Diese Doktorarbeit stellt die erste umfassende Studie auf Transkriptionsebene zur Anpassung an abiotischen Stress in einer Kelp-Art aus der Arktis, *Saccharina latissima*, dar. Hauptziel dieser Arbeit ist die Untersuchung der molekularen Prozesse der physiologischen Akklimatisation an mehrere abiotische Stressoren.

Junge *S. latissima* Sporophyten sind für 8 Stunden, 24 Stunden und 2 Wochen in multifaktoriellen Experimenten verschiedenen Kombinationen von photosynthetisch aktiver Strahlung (PAR), UV Strahlung und Temperatur ausgesetzt worden. Zur Erfassung der physiologischen Fitness ist die photosynthetische Effizienz von Photosystem II (Fv/Fm) am Anfang sowie am Ende der Experimente gemessen worden. Als Grundlage für funktionelle genomische Studien der Stressakklimatisation wurde aus der RNA der exponierten Sporophyten eine cDNA Datenbank erstellt. Darauf folgend wurden mit Hilfe von Microarray Hybridisierungen Genexpressionsprofile unter abiotischen Stress erstellt. Zum Vergleich molekularer Mechanismen von Kurzzeit- und Langzeit-Anpassung ist ein 24 stündiger Versuch und ein 2 wöchiger Versuch auf transkriptionaler Ebene untersucht worden. Abschließend wurden vergleichend Genexpressionprofile in Freiland und Kulturmaterial untersucht, um die Auswirkung verschiedener Wachstumsbedingungen auf die Anpassung an UV Strahlung und Temperatur zu erforschen.

Die erstellte cDNA Datenbank besteht aus 400,503 ESTs, welche zu 28,803 Contigs assembliert wurden. Mit verschiedenen Methoden konnten über 10,000 Contigs mutmaßliche Funktionen oder orthologische Beziehungen zugeordnet werden. Ein Vergleich der Sequenzen mit dem Genom von *E. siliculosus* zeigte, dass die cDNA Datenbank ca. 70% des funktionellen Genoms abdeckt. Die cDNA Datenbank reflektiert somit einen repräsentativen Teil des Genoms und kann als weitgehend kompletter Genkatalog dieser Art angesehen werden. Sie stellt damit eine hervorragende Basis für weitere molekulargenetische Untersuchungen dar.

Saccharina latissima reagierte auf abiotischen Stress mit einer Vielzahl an Änderungen auf transkriptionaler Ebene. Der Faktor Temperatur hatte einen geringeren Einfluss auf metabolische Prozesses als der Faktor Licht. Zwei allgemeine Temperatureffekte wurden beobachtet, einerseits die Induktion von Genen des Glycin-, Serin- und Threonin-Stoffwechsels bei niedrigen Temperaturen, andererseits die Repression von Transkripten des biosynthetischen und katabolischen Kohlenhydrat-Stoffwechsels bei hohen Temperaturen. Hohe photosynthetisch aktive Strahlung führte zur Induktion katabolischer Prozesse und zur Induktion von Hitzeschock-Proteinen sowie Proteinen mit antioxidativer Funktion. Die Kombination der Stressfaktoren hohe photosynthetisch aktive Strahlung, Temperatur und UV-Strahlung führte zu interaktiven Effekten in der Photosynthese und der Genexpression. Am schädlichsten war für die Alge die Kombination von hoher Lichtintensität und der höchsten,

getesteten Temperatur, 17°C. Unter diesen Bedingungen wurden wichtige Stoffwechselprozesse, wie z.B. die Photosynthese und der Kohlenhydrat- und Aminosäure-Stoffwechsel gehemmt. Akklimatisation an hohe photosynthetisch aktive Strahlung und tiefe Temperaturen führte zu verstärkter Induktion des Glycin-, Serin- und Threonin-Stoffwechsels, möglicherweise als Konsequenz eines erhöhten Bedarfes an Glutathion, einem wichtigen Co-Faktor von Enzymen, die am Abbau reaktiver Sauerstoffspezies (ROS) beteiligt sind.

Die Bildung reaktiver Sauerstoff-Spezies stellt ein zentrales Element der abiotischen Stressantwort dar. Die nachgewiesene Regulation diverser ROS-detoxifizierender Enzyme als Reaktion auf Temperatur- und Lichtstress zeigt, dass ROS eine wichtige Rolle spielen in der Anpassung an abiotischen Stress in *S. latissima*. Die Untersuchungen ergaben erstmals Hinweise auf die Existenz Kompartiment-spezifischer ROS-Abbau-Mechanismen bei *S. latissima*. Ferner wurde bei der Temperaturanpassung, und auch bei der Anpassung an kombinierte Faktoren, wie z.B. hohe photosynthetisch aktive Strahlung im Zusammenspiel mit hohen Temperaturen eine komplexe Regulation von Hitzeschock Proteinen beobachtet.

Langzeit- sowie Kurzzeitanpassung an UV-Strahlung führte zu einer erhöhten Regulation von photosynthetischen Komponenten, wie z.B. Thylakoid-Proteinen, Lichtsammelkomplex-Proteinen und Proteinen der beiden Photosysteme. Genexpressionsanalysen zeigten, dass Photosystem II anfälliger gegenüber UV Strahlung ist als Photosytem I. Die Reparaturmechanismen von UV-induzierten Schäden am Photosystem II scheinen zu einer Erhöhung des Transkriptpools von Photosystem II Komponenten zu führen. Ein herausragendes Merkmal war die verstärkte Induktion des Vitamin B_6 Stoffwechsels nach Kurzzeit- und Langzeitbestrahlung mit UV, welche eine wichtige Rolle in der Anpassung an UV Strahlung in *Saccharin latissima* zu spielen scheint.

Erhöhte Regulation von DNA-Replikation und DNA-Reparatur wurde nur nach Kurzzeitbestrahlung mit UV nachgewiesen. Hierbei wurde ein Zusammenspiel der DNA-Reparaturmechanismen Photoreaktivierung, homologe Rekombination und Nukleotidexzisionsreparatur beobachtet. Dies weist auf eine hochentwickelte Regulation der verschieden DNA-Reparaturprozesse hin. Da nach dem 2-Wochen-Langzeitversuch keine verstärkte Regulation des DNA Stoffwechsels mehr beobachtet wurde, scheint *S. latissima* in der Lage zu sein, sich an UV-Strahlung anzupassen und durch UV-Strahlung entstandene DNA-Schäden zu überwinden.

Der abschließende Vergleich von Genexpressionsprofilen in Kultur- und Freilandmaterial von *Saccharina latissima* zeigte Unterschiede in der Genregulation auf. Hierbei wurden große Unterschiede in der Anzahl der regulierten Gene beobachtet. Andererseits waren sich aber die allgemeinen Effekte von UV-Strahlung und die Schädigung von Photosynthese und DNA ähnlich. Dies heißt, dass Laborexperimente gut geeignet sind um grundsätzliche molekulare Anpassungsmechanismen an abiotischen Stress zu untersuchen. Im Einzelnen zeigte sich eine höhere Anfälligkeit von Freiland-Sporophyten gegenüber UV-Strahlung und eine erhöhten Grad an oxidativem Stress bei einer Temperatur von 12°C, wohingegen Kulturmaterial größere Anstrengungen unternehmen muss, um sich einer Temperatur von 2°C anzupassen. Dieses Ergebnis ist weitgehend auf die unterschiedlichen Wachstumstemperaturen (zwischen -3°C und 1°C für Freiland-Sporophyten und 10°C für Kulturmaterial) zurückzuführen. Jedoch lässt dieses Ergebnis auch darauf schließen, dass die Kälteanpassung von Freilandmaterial zu Änderungen im Stoffwechsel führt, die zwar eine erhöhte Stressresistenz bei niedrigen Temperaturen zur Folge hatte, aber gleichzeitig auch zu einer höheren Anfälligkeit gegenüber einer Temperatur von 12°C führte.

Diese Doktorarbeit zeigt die Komplexität der molekularen Anpassung an abiotischen Stress in *Saccharina latissima*. Die in dieser Arbeit erlangten molekularen Daten erweitern unser Verständnis in Bezug auf die Stressantwort einer Kelp-Art, und stellen ein gute Grundlage für weitere molekulare Studien dar. Des Weiteren zeigt diese Arbeit wie wichtig es ist, Interaktionen zwischen zwei oder mehreren abiotischen Stressfaktoren auf physiologischer sowie molekularer Ebene zu untersuchen. Die Ergebnisse der vergleichenden Genexpressionsstudie zeigen wie das Wachstum unter den verschiedenen Expositionsbedingungen von den molekularen Prozessen der Stressanpassung abhängt, und unterstreichen die Bedeutung von Versuchen mit Freilandmaterial bei der Vorhersage der Folgen veränderter Umweltbedingungen im Freiland.

ABBREVIATIONS

~	Approximately
%	Percent
°C	Degree Celsius
<	Smaller than
>	Greater than
ANOVA	analysis of variance
APX	Ascorbate peroxidase
Asa	Ascorbate
bp	Basepair
cDNA	Complementary deoxyribonucleic acid
CDPK	Calcium-dependent protein kinase
CPDs	Cyclobutane-pyrimidine dimers
cRNA	Complementary ribonucleic acid
cy-3	Cyanine-3
cy-3-CTP	Cyanine-3-cytidine triphosphate
cy-5	Cyanine-5
cy-5-CTP	Cyanine-5-cytidine triphosphate
DNA	Deoxyribonucleic acid
EC	Enzyme code
EST	Expressed sequence tag
*F*v/*F*m	Maximum quantum yield of photosystem II
GR	Glutathione reductase
GSH	Glutathione
GO	Gene Ontology
h	Hours
HP	High photosynthetically active radiation
HPAB	High photosynthetically active radiation including UV radiation (280-700 nm)
Hsps	Heat shock proteins
kb	Kilobase
kDa	Kilodalton
KEGG	Kyoto Encyclopedia of Genes and Genomes
KOBAS	KEGG Orthology Based Annotation System
LHC	Light-harvesting complex
LP	Low photosynthetically active radiation
LPAB	Low photosynthetically active radiation including UV radiation (280-700 nm)
M	Mole
MAP	Mitogen-activated protein
mM	Millimolar
mRNA	messenger ribonucleic acid
nm	Nanometer
p	Probability
PAM	Pulse Amplitude Fluorometer
PAR	Photosynthetically active radiation (400-700 nm)
PES	Provasoli enriched seawater
PS I	Photosystem I
PS II	Photosystem II
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SOD	Superoxide dismutase
tRNA	Transfer ribonucleic acid
Trx	Thioredoxin
UTRs	Untranslated regions
UVR	Ultraviolet radiation (220-400 nm)
UV-A	Ultraviolet radiation A (320-400 nm)
UV-B	Ultraviolet radiation B (280-320 nm)
w	weeks
W	Watt [SI Unit]
µ	Micro
µg	Microgram
µmol	Micromole

LIST OF PUBLICATIONS AND DECLARATION OF CONTRIBUTIONS

This thesis is based on the following papers, referred to by their Roman numbers:

I. **Heinrich S**, Frickenhaus S, Glöckner G, Valentin K (2012). A comprehensive cDNA library of temperature stressed *Saccharina latissima* (Phaeophyceae). European Journal of Phycology, in press

II. **Heinrich S**, Frickenhaus S, John U., Valentin K, Wiencke, C. (2012). Transcriptomic Analysis of Acclimation to Temperature and Light Stress in *Saccharina latissima* (Phaeophyceae). PloS ONE, under review

III. **Heinrich S**, Frickenhaus S, Valentin K, Wiencke, C. Interactive effects of temperature, high photosynthetically active radiation, and UV radiation on gene expression in *Saccharina latissima* (Phaeophyceae). Plant Physiology, submitted

IV. **Heinrich S**, Frickenhaus S, Valentin K, Wiencke, C. Comparative gene expression in field and cultivated sporophytes of *Saccharina latissima* (Phaeophyceae) exposed to different radiation and temperature conditions. Manuscript in preparation

Declaration of contributions

Publications I-IV were initiated, conceptualized and conducted by Sandra Heinrich in close cooperation with Klaus Valentin and Christian Wiencke. Stephan Frickenhaus provided assistance in bioinformatics analysis and produced the R-figures. The data were interpreted and the manuscripts written by Sandra Heinrich in discussion with the respective co-authors.

1. Introduction

1.1 Marine macroalgae

Marine macroalgae are distributed on rocky coastal shores throughout the world`s ocean (Reiskind *et al.*, 1989; Lüning, 1990). They serve a multitude of ecosystem functions, and are therefore commonly recognized as a key species in helping to structure the biodiversity of coastal ecosystems (Bischof *et al.*, 2006; Tait and Schiel, 2011). Marine macroalgae play a critical role in primary production, and account for up to 10% of the global oceanic primary production, even though they cover only a small percentage of the area of the world's oceans (Smith, 1981; Charpy-Roubaud and Sournia, 1990). They furthermore constitute important habitat-forming components of rocky shore ecosystems (Duggins *et al.*, 1990; Bertness *et al.*, 1999; Pearson *et al.*, 2010). In addition to their value to ecosystem function, they are of significant economic importance (Häder and Figueroa, 1997). They comprise a marine source for food (Mabeau and Fleurence, 1993; Fleurence, 1999), alginic acid (Crépineau *et al.*, 2000), as well as for biochemical compounds and pharmaceuticals (Waaland *et al.*, 2004; Roeder *et al.*, 2005).

The distribution of macroalgae is limited by abiotic factors as well as by biological interactions, both on a biogeographic scale as well as on a local one. The geographic distribution of marine macroalgae is primarily constrained by their temperature demands for growth and reproduction, as well as by temperature tolerance of the different life cycle stages of species (van den Hoek, 1975, 1982, 1982). A major factor determining depth distribution of macroalgae is their ability to resist high solar radiation; studies have shown that species' sensitivity to short wave UV radiation plays an important role in shaping the vertical distribution of marine macroalgae (Markager and Sand-Jensen, 1992; Hanelt *et al.*, 1997; Bischof *et al.*, 1998; Hanelt, 1998; Wiencke *et al.*, 2006). Other abiotic factors influencing depth distribution are e.g. tolerance to desiccation and salinity (Schonbeck and Norton, 1978; Dring and Brown, 1982; Davison and Pearson, 1996). It should however also be noted that biological interactions, such as interspecific competition and grazing, also have an effect on the vertical distribution patterns of macroalgae (Chapman, 1974; Hruby, 1976; Lubchenco, 1980).

1.1.1 Laminariales

Macroalgae of the order Laminariales (= "kelp") are distributed from cold to temperate regions of the Arctic to the Antarctic (Lüning, 1990; Lane *et al.*, 2006). Phylogenetic studies on the Laminariales suggest that ancestors of the genus *Laminaria* have originally evolved in the North Pacific and radiated to the North Atlantic and the Arctic during the latest Pliocene or Pleistocene (Lane *et al.*, 2006; Adey *et al.*, 2008).

Members of the Laminariales exhibit a very high degree of morphological and anatomical specialisation among all algae (Saunders and Druehl, 1992). Their life history is characterized by a complex haplo-diplophasic life cycle, in which microscopic haploid gametophytes alternate with macroscopic diploid sporophytes (Sauvageau, 1915), that in some species can reach a length up to 60m (Cribb, 1954). Many of the kelps species are annuals, but some are perennials, e.g. *Laminaria hyperborea*, which has a lifespan up to 20 years (van den Hoek *et al.*, 1995).

Kelps form huge submarine forests ("kelp beds"), which are structurally complex and highly productive components of cold-temperate oceans (Steneck *et al.*, 2002; Bischof *et al.*, 2006; Lane *et al.*, 2006). Extensive kelp forests represent some of the most productive marine ecosystems in temperate regions (Phillips *et al.*, 2011), and are the largest biogenic structures found in benthic marine systems (Dayton, 1985). These structures offer food, protection from predators and nursery grounds for e.g. fishes and invertebrates (Duggins *et al.*, 1990; Steneck *et al.*, 2002; Carlsen *et al.*, 2007). Little of kelp-produced biomass is actually consumed directly by herbivores; it rather enters the detritus based food web and therefore represents a significant source of nutrition for coastal marine ecosystems (Duggins *et al.*, 1989; Kaehler *et al.*, 2006; Bartsch *et al.*, 2008). In addition, kelp canopies profoundly alter the local environment through wave dampening and reduction of alongshore currents. Their influence in the flow of water has further downstream consequences on such associated processes as sedimentation and coastal erosion (Jackson and Winant, 1983; Jackson, 1984; Eckman *et al.*, 1989).

1.1.2 *Saccharina latissima*

The perennial kelp *Saccharina latissima* (Lane *et al.*, 2006) (formerly *Laminaria saccharina* L. (Lamour)) is distributed in a circumpolar fashion across the northern hemisphere, and is common from polar to temperate coastal waters (Bolton *et al.*, 1983; Borum *et al.*, 2002). The wide geographic distribution of this species suggests a high capacity for climatic adaption. The presence of dense stands of *S. latissima* in polar regions indicates that this species possesses a high acclimation potential to cold temperature and low light (Kirst and Wiencke, 1995; Borum *et al.*, 2002).

Growth sites of *S. latissima* are both, clear and turbid coastal waters, from the intertidal down to 30 m depth; consequently that species is exposed to a wide range of temperature and light conditions (Gerard, 1988). Growth in *S. latissima* is limited by light on a seasonal basis and light availability often determines the maximum depth for survival. Hence the ability to maintain a positive photosynthesis and growth under a variety of environmental conditions is important for the broad distribution of this species (Lüning, 1979, 1990).

The wide latitudinal and vertical distribution of this species is thought to be associated with ecotypic differentiation of populations, with respect to light and temperature. Different light and temperature traits were reported for sporophytes and gametophytes of *S. latissima* from the Long Island Sound (USA) and the Atlantic coast from Maine (USA) (Gerard, 1988; Gerard and Dubois, 1988; Gerard, 1990) as well as for gametophytes from Spitsbergen (Norway) and Helgoland (Germany) (Müller *et al.*, 2008). The variation in light- and temperature related traits is suggested to have a genetic basis, but until now molecular studies on ecotypic variation are missing.

1.1.3 Macroalgae in Arctic environments

Macroalgae of the polar regions are subjected to strong seasonal changes in physical and chemical conditions, e.g. an extreme seasonality of light conditions, accompanied by low water temperatures (Kirst and Wiencke, 1995; Zacher *et al.*, 2009). In the Arctic, annual solar radiation is 30-50% lower than in temperate to tropical regions, and the polar night lasts approximately 2-4 month depending on the latitude (Lüning, 1990). Darkness and the sea cover cause dim light conditions. Light measurements in Spitsbergen (Norway) below a sea ice cover of 1m thickness showed low maximal photon flux densities of 6.5 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR, 400-700nm) (Hanelt *et al.*, 2001). Studies in the Arctic fjord Kongsfjorden showed, that after the break-up of the sea ice, low light conditions are replaced by high light conditions; due to the clear water PAR and UV radiation penetrating deeply into the water column, and PAR can increase up to 600 µmol photons m^{-2} s^{-1} in 4m depth (Hanelt *et al.*, 2001; Bischof *et al.*, 2002; Svendsen *et al.*, 2002; Brey, 2009). The presence of ice not only influences light conditions, but also can lead to mechanical damage, subsequently influencing macroalgal zonation patterns along coastal shores (Barnes, 1999; Wiencke *et al.*, 2004; Zacher *et al.*, 2009).

Macroalgae in high latitude regions contribute strongly to coastal primary productivity and energy fluxes, exceeding or equalling the production of primary producers in more temperate systems (Gómez *et al.*, 2009). Despite the constant low temperature and the extreme and unstable underwater light conditions, rocky coastal shores of polar regions exhibit dense stands of perennial macroalgae (Borum *et al.*, 2002). The occurrence and the high abundance of these mainly brown algae dominated communities suggests, that they possess a high adaptional potential for low light and low temperatures (Kirst and Wiencke, 1995). Cold adapted species developed mechanisms, such as changes in gene expression in order to maintain sufficient rates of enzyme-catalyzed reactions and modifications within the thylakoid membrane system, affecting photosynthetic electron transport, to overcome the constraints of exposure to low temperature (Ensminger *et al.*, 2006; Gómez *et al.*, 2009).

1.2 Effects of abiotic stress on macroalgae

1.2.1 Single stress effects: Temperature and radiation

For sessile organisms, it is of particular importance to acclimate and adapt to environmental changes for maintaining cellular function. In plants, extrinsic stress resulting from changes in abiotic factors, e.g. light and temperature is regarded as the most important stress agent (Qureshi *et al.*, 2007). In general, enzyme activity decreases with declining temperatures, therefore low temperatures significantly alter plant metabolism, physiology and productivity (Perelman *et al.*, 2006; Kaplan *et al.*, 2007). Low temperature leads to reduced activity of the Calvin cycle, resulting in a decrease of production for the final electron acceptor $NADP^+$, which may lead to electron transfer from reduced ferredoxin to oxygen and finally to the formation of reactive oxygen species (ROS) (Pfannschmidt, 2003). High temperatures cause degradation and dysfunction of proteins, an uncoupling of pathways resulting in the formation of ROS, which – in turn - induces lipid peroxidation (Panchuk *et al.*, 2002; Dring, 2006; Lesser, 2006; Timperio *et al.*, 2008). Investigations on temperature effects in macroalgae has demonstrated that temperature influences e.g. growth rates, enzyme activity, photosynthesis and, as a consequence, biogeography of macroalgae (Fortes and Lüning, 1980; Bolton and Lüning, 1982; Lüning, 1984; Davison and Davison, 1987; Davison *et al.*, 1991; tom Dieck, 1992; Machalek *et al.*, 1996).

Solar radiation is essential for carbon assimilation and oxygen production in photoautotrophic organisms, however, when irradiances exceed the energy requirements for photosynthesis, photosynthetic activity decreases (Krause and Weis, 1991). The impact of changing irradiance and light quality on physiology of macroalgae has been studied extensively. Intense photosynthetically active radiation leads to photoinhibition and destruction of photosynthetic components (Franklin and Forster, 1997; Hanelt *et al.*, 1997; Altamirano *et al.*, 2004). Furthermore excessive light causes photo-oxidative stress through an over-reduction of the photosynthetic electron transport chain, which leads to the formation of reactive oxygen species (ROS), e.g. superoxide radicals and hydrogen peroxides (Bischof *et al.*, 2003; Janknegt *et al.*, 2008; Bischof and Rautenberger, 2012). Exposure to UV radiation leads to manifold effects on algal metabolism and physiology. In particular, photosynthesis, growth and reproduction are negatively affected; furthermore it disturbs normal DNA base pairing and leads to the formation of ROS (Franklin *et al.*, 2003; Bischof *et al.*, 2006).

1.2.2 Multiple stress effects

In the natural environment, plants are seldom subjected to a single stress factor, but rather to a multitude of abiotic and biotic factors (Alexieva *et al.*, 2003). Stressors can act either synergistically, in which case their combined effect is larger than predicted from the sizes of the responses to each stressor alone; or antagonistically, where the cumulative effect is smaller (Folt *et al.*, 1999). While single effects of abiotic factors on seaweed physiology are well studied, very few projects focus on combined interactions of various factors. To date there are, however, some studies on the interaction of UV radiation and temperature.

Studies on interactive effects of temperature and UV-B radiation on macroalgal photosynthesis has shown that the photosynthetic performance of, e.g. *Ulva clathrata*, *Alaria esculenta* and *Palmaria palmata* was less impaired by UV-B radiation at high than at low temperatures (Van De Poll *et al.*, 2002; Rautenberger and Bischof, 2006; Fredersdorf *et al.*, 2009). Pakker *et al.* (2000) investigated the temperature influence on repair of UV-B induced DNA damage in *Palmaria palmata*, where DNA repair rates increased with rising temperatures. Two other studies have focused on the interactive effects of temperature and UV radiation on germination in *Fucus gardneri* and *Alaria marginata*; germination rates of both species increased with rising temperatures as long as the temperatures did not approach upper thermal maxima (Hoffman *et al.*, 2003; Müller *et al.*, 2008).

Despite these studies, we continue to lack information concerning interactive effects of combined stressors in macroalgae. Further studies are needed to investigate in more detail how changing environmental factors, such as enhanced CO_2 concentrations, changing salinity and enhanced UV radiation due to ozone depletion interact with the factor of global warming.

1.2.3 Reactive oxygen species

Reactive oxygen species (ROS) are basally produced in plants as by-products of aerobic metabolic processes in mitochondria, chloroplasts and peroxisomes (Apel and Hirt, 2004).

ROS cause oxidative damage in terms of denaturation of nucleic acids, proteins, polysaccharides and lipids, and are removed by cell compartment specific non-enzymatic and enzymatic scavenging systems (Asada, 1997; Mittler, 2002). Non-enzymatic antioxidants include the major cellular redox buffers ascorbate and glutathione, whereas enzymatic ROS detoxification includes the conversion of superoxide radicals into hydrogen peroxide and oxygen by superoxide dismutase, and subsequent elimination of hydrogen peroxide in the ascorbate–glutathione cycle by ascorbate peroxidase and glutathione reductase (Noctor *et al.*, 1998; Asada, 1999; Apel and Hirt, 2004).

Studies on effects of environmental stress in macroalgae have demonstrated, that the increased formation of ROS is a central element of abiotic stress response (Dring, 2006). Increased activity of ROS scavenging enzymes in macroalgae has been reported following exposure to UVR stress, copper, low temperature and desiccation (Collén and Davison, 2001; Aguilera *et al.*, 2002; Burritt *et al.*, 2002; Bischof *et al.*, 2003; Contreras *et al.*, 2009; Contreras-Porcia *et al.*, 2011).

ROS are however not only potentially harmful, but are also part of a subtle network of signalling reactions (Noctor, 2006). ROS as well as the redox state of several regulatory redox-reactive key molecules, such as thioredoxin and glutathione, are signals that regulate expression of photosynthesis-related genes (Pfannschmidt, 2003; Foyer and Noctor, 2005). Furthermore ROS function as second messengers in signal transduction cascades, regulating processes such as mitosis and cell death (Foyer and Noctor, 2005). Additionally, many heat shock proteins are thought to be tightly associated to ROS. In plants it has been suggested that sensing of ROS functions via redox-sensitive transcription factors, which subsequently activate Hsp expression (Mittler *et al.*, 2004; Timperio *et al.*, 2008).

1.3 Global environmental changes

There is evidence that the global climate is currently changing. Analysis of temperature records revealed a strong linear warming trend over the 50 years from 1956 to 2005. Climate change is caused by both, natural and anthropogenic drivers. However, a major reason for the observed warming is the anthropogenic emission of gases, in particular carbon dioxide, into the atmosphere, leading to an increase in the planets global temperature. Until now several effects of warming have been observed, e.g. decreases in snow cover and Northern Hemisphere sea ice extent, increases in soil temperature, sea level rise and glacier melt (IPCC, 2007). The future high scenario of the Fourth Assessment Report of the United Nations Intergovernmental Panel on Climate Change predicts a global average surface warming in the range of 2.4–6.4°C by 2100, thereby warming is expected to be greatest at most high northern latitudes and least near Antarctica (IPCC, 2007). Terrestrial areas warm faster than ocean water masses, nevertheless warming occurs in both systems (ACIA and Committee, 2005). Since 1950 mean annual sea surface temperatures in the North Atlantic have already risen by more than 1.5°C, which is more than twice of the global average (Merzouk and Johnson, 2011).

Stratospheric ozone content has significantly diminished during the 50 years due to growing emissions of synthetic chlorofluorocarbon molecules. The ozone layer absorbs the most energetic fraction, UV-C radiation, as well as parts of the UV-B wavelength range (Rowland, 2006). Hence depletion of ozone has caused increases in UV-radition at the earth`s surface, which are particularly pronounced at higher latitudes (Kerr and McElroy, 1993; Madronich *et al.*, 1998). Over the years, a strong decrease in the ozone density, especially in the polar regions, has been reported (Karsten et al., 2001). A recent study demonstrated that the degree of chemical ozone destruction observed over the Arctic in early 2011 was, for the first time, comparable to that in the Antarctic (Manney *et al.*, 2011). In the worst case scenario, an ozone depletion over the Arctic up to 20 % until 2020 is forecasted (WMO, 2006).

Future ecological effects of global environmental changes still raise considerable concerns (Hoffman *et al.*, 2003; Harley *et al.*, 2006). There is consensus that coastal marine ecosystems are endangered by anthropogenic global climate change (IPCC, 2001; Helmuth *et al.*, 2006). Abiotic factors predominantly determine vertical and geographical distribution of

benthic macroalgae. It is likely that the increase of UV radiation and sea surface temperatures will influence their distribution patterns, especially in the Arctic, which is subjected to strong environmental changes. Latitudinal shifts in species distribution of macroalgae as ecological responses to climate changes have been already observed (Simkanin *et al.*, 2005; Lima *et al.*, 2007; Hawkins *et al.*, 2009). Likewise, the depth distributions of seaweeds may be altered due to changes in the radiation regime.

1.4 Functional genomics

Increasing knowledge of genes and gene products due to genome-based technologies has significantly contributed to the understanding of algal ecology and evolution (Grossman, 2005). Genomic approaches, such as functional and marine ecological genomics, combine molecular biology, computing sciences and statistics (Dupont *et al.*, 2007).

Presently, analysis of whole genomes is rapidly becoming a trend that allows remarkable insights into fields such as comparative evolution, physiology, and developmental biology (Waaland *et al.*, 2004). A more cost-effective genomic resource that can be developed for almost any organism is an expressed sequence tag (EST) library, also called cDNA library. This provides a robust sequence resource for gene and genomic analysis, especially in non-model organisms that lack other sequence resources (Bouck and Vision, 2007). Furthermore, EST libraries can be exploited for gene discovery, genome annotation, expression profiling, comparative genomics as well as microarray design (Rudd, 2003; Dupont *et al.*, 2007). Microarray technology, first applied in the mid 1990s, has become a routine and essential tool not only for gene expression profiling, but also for gene discovery, detection of single nucleotide polymorphism (SNPs) and comparative genomic hybridization (Schena *et al.*, 1995; Li *et al.*, 2002; Gadgil *et al.*, 2005).

Despite the ecological and economic importance of marine benthic macroalgae, their molecular biology is poorly understood. Up until now only the genome of one macroalga, the brown algal species *E. siliculosus*, has been fully sequenced (Cock *et al.*, 2010). The availability of the *Ectocarpus* genome gave rise to several new studies, e.g. of the molecular basis of cell wall polysaccharide metabolism (Michel *et al.*, 2010), mannitol synthesis (Rousvoal *et al.*, 2011), developmental patterning (Le Bail *et al.*, 2011), and inter-strain variation (Dittami *et al.*, 2011). Currently, only two microarray-based studies on gene expression under abiotic stress in macroalgae are published. Collén *et al.* (2007) studied transcriptional changes in *Chondrus crispus* after exposure to different abiotic stresses; in their study 25% of genes exhibited transcriptional changes. In *Ectocarpus siliculosus*, almost 70% of the expressed genes featured significant changes in transcript abundance in response to stress (Dittami *et al.*, 2009). These studies indicate that macroalgae respond to stress with a multitude of transcriptional changes. Undoubtedly more studies are needed to expand our knowledge of molecular mechanisms underlying the physiological acclimation to abiotic stress in macroalgae.

1.5 Aim of the thesis

Future global environmental changes will likely influence zonation, biogeographic distribution patterns, and primary productivity of ecologically and economically important kelp species. During past years many physiological studies have been performed on effects of a single abiotic stress in kelp, whereas very few studies have focused on the interactive effects of multiple stresses. So far, no study is available on the molecular processes underlying physiological acclimation to abiotic stress factors in kelps. For that reason, the purpose of the present thesis was to investigate molecular mechanisms underlying the physiological acclimation to multiple abiotic stresses in *S. latissima* from the Arctic (Spitsbergen). The molecular data obtained by this study will be a starting point for further investigations using molecular approaches in kelps. Furthermore, the results of this thesis will help to obtain important insights into the characterization of molecular processes underlying physiological acclimation as well as to improve our understanding of stress responses in a particular kelp species.

The first aim of this thesis was to establish a cDNA library for subsequent functional genomic studies on mechanisms and pathways involved in stress acclimation to multiple stressors. Microarray hybridizations were used to determine key genes involved in molecular responses to high PAR, UVR, and temperature stress. Two different stress exposure durations, 24 hours and 2 weeks, were applied to distinguish molecular mechanisms of short-term versus long-term acclimations to stress. Furthermore, a comparative approach, investigating gene expression profiles in laboratory and field grown sporophytes, was carried out in order to help elucidate interactive effects of UVR, temperature and growth conditions in laboratory versus field material.

2. Methodological aspects

The following chapter provides a brief overview of the material and methods applied in the experimental portion of this thesis. Further details, in particular regarding sampling, experimental conditions, experimental processing and analysis techniques are detailed in the respective publications.

2.1 Algal material

Culture material

Young sporophytes were raised from stock gametophytes cultures of *Saccharina latissima*, originally established from spores of fertile sporophytes collected by SCUBA diving in Kongsfjorden (79°N; 11°E; Svalbard, Norway, AWI culture numbers: 3123, 3124). Male and female gametophytes were fragmented together, transferred to Petri dishes filled with Provasoli enriched seawater (PES) (Starr and Zeikus, 1993) and cultured at 10 +/- 1°C and 30 µmol photons m^{-2} s^{-1} white light at 18h light: 6h dark period. Developing sporophytes were transferred after 2 weeks to aerated 5l culture bottles and grown in PES until they reached a size of 5-7 cm.

Field material

Algal material was collected May 2009 directly after the sea ice breakup in the Kongsfjorden (79°N; 11°E; Svalbard, Norway) Sporophytes of *Saccharina latissima* with a size of 5-7 cm were collected by SCUBA diving from 8 m water depths. Algae were then transported back to the laboratory, cleaned of epiphytes, and kept for 48h in running seawater at approximately 4°C under low light (10 µmol photons m^{-2} s^{-1}) prior to the exposure experiments.

2.2 Exposure to artificial defined stress conditions

Young sporophytes were exposed to six different radiation conditions and four temperatures (Fig. 1). To distinguish the effects of different wavelengths, the experimental units were covered with filter foils permeable to wavelengths of: (1) 400–700 nm for photosynthetically active radiation (PAR) treatments; (2) 320–700 nm for PAR+UVA treatments; and (3) 295–700 nm for PAR + UVA + UVB treatments. PAR was provided by Osram daylight fluorescent tubes, and measured using a LI- 250 light meter (LI-COR, Lincoln; USA). UV radiation was generated by three fluorescent tubes and measured with a Solar Light PMA-2100 (Solar Light; PA, USA). For the exposure experiments sporophytes were placed into white PVC boxes and covered with the respective filter foils. Aeration was provided through the whole experimental duration of 8h, 24h and 2 weeks. All experiments were conducted with five biological replicates.

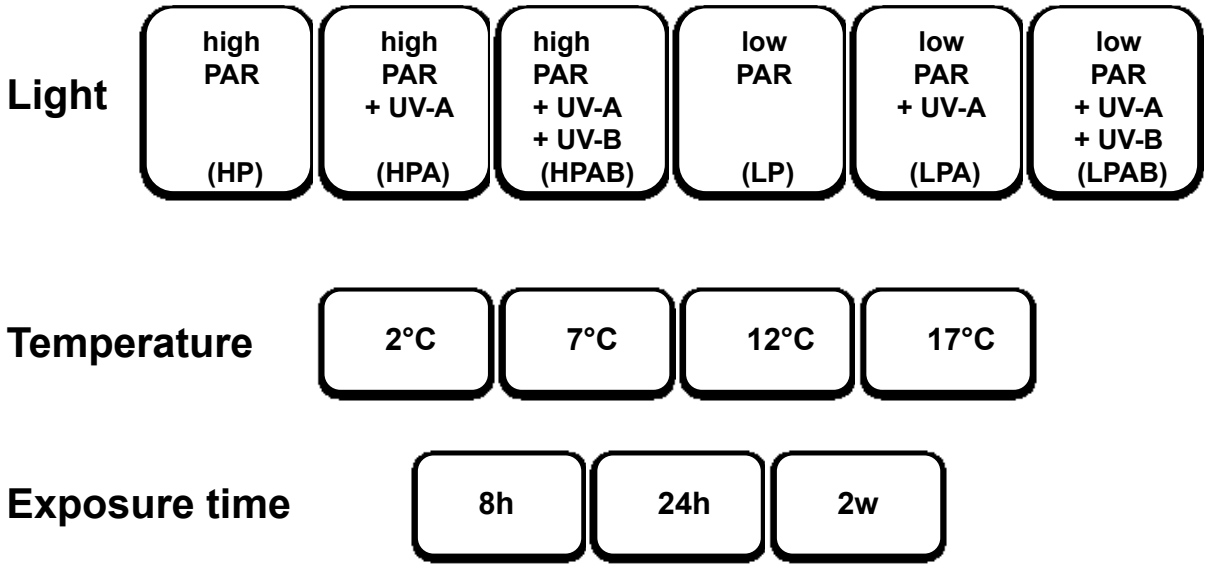
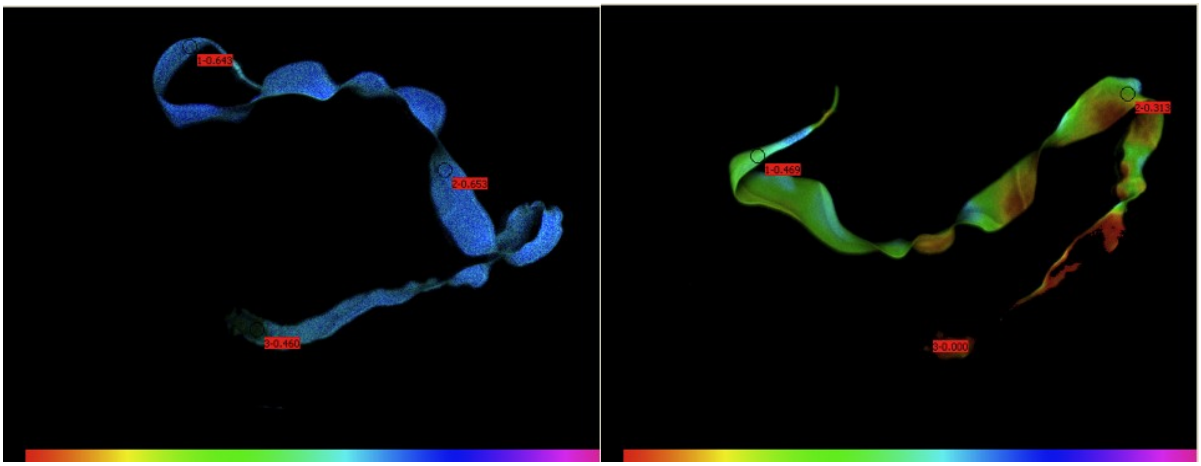


Figure 1. Overview of multifactorial exposure experiments.

2.3 Photosynthetic measurements

Photosynthetic performance of sporophytes under the applied stresses was determined by measuring maximum quantum yield of photosystem II (Fv/Fm) using an Imaging pulse-amplitude fluorometer (Maxi Imaging PAM; Walz, Effeltrich, Germany). Measuring with the PAM is a non-invasive method; the Maxi Imaging-PAM furthermore provides a fast assessment of photosynthetic activity across the whole sporophytes (Fig. 2). Fv/Fm measurements provide an estimate of the maximum quantum efficiency of PSII and indicate the efficiency of energy transfer from the light harvesting complexes to the reaction centre of PS II. A change in Fv/Fm is due to an alteration in non photochemical quenching, decreasing Fv/Fm which indicates the occurrence of photoinhibitory damage, therefore it is a commonly used parameter in stress investigating experiments (Maxwell and Johnson, 2000). The physiological basis of chlorophyll fluorescence and fluorescence measurements by saturation pulse method is extensively reviewed by Krause and Weis (1991), Schreiber *et al.* (1994) and Baker (2008).



A B

Figure 2. Images of maximum quantum yield of a *S. latissima* sporophyte generated by the Maxi Imaging Pam before (A) and after 24 hours (B) of simultaneous exposure to low PAR and UVR.

2.4 Molecular methods

RNA Isolation

Macroalgae exhibit high amounts of polysaccharides (Wang *et al.*, 2005; Varela-Alvarez *et al.*, 2006) and phenolic compounds (Lane *et al.*, 2006; Pearson *et al.*, 2006), making RNA and DNA isolation difficult. A novel protocol had to be established for RNA extraction because standard procedures proved to be inapplicable to brown algae; they did not produce sufficient amounts or qualities of RNA needed for downstreaming applications, e.g. cDNA synthesis and microarray hybridization. The developed protocol is a combination of a CTAB extraction, followed by the use of a commercial RNA isolation kit. The extraction protocol was tested successfully in several brown algae (e.g. *Laminaria solidungula*, *Alaria esculenta* and *Fucus serratus*).

Establishment of the cDNA library

RNA from differently treated sporophytes was pooled for cDNA library construction. From the total RNA, poly $(A)^+$ RNA was prepared, thus also minimizing potential bacterial contamination. cDNA synthesis was primed with an N6 randomized primer. Normalization was carried out by one cycle of denaturation and reassociation of the cDNA. For 454-sequencing (reviewed by Mardis, 2008) the cDNA in the size range of 500–700 bp was eluted from a preparative agarose gel. 454-sequencing of the cDNA was conducted according to the manufacturer's protocols (Roche, Branford, USA). Assembly of the raw reads was done using the Newbler assembler (Roche) with the default values. Assembled cDNA, i.e. the contigs, were compared against the Swiss-Prot protein knowledgebase (http://www.uniprot.org/) and the NCBI non-redundant protein database (http://blast.ncbi.nlm.nih.gov/Blast.cgi, NCBI-nr), using the BLASTX algorithm with an E-value cut-off of $10e^{-7}$. Sequences were mapped according to Gene Ontology (GO), and searched by Interpro scan for functional annotations of protein domains. All Sequence annotation steps were done using the Blast2GO analysis tool (Conesa *et al.*, 2005).

Microarray design and hybridization

Microarrays slides were designed with Agilent's eArray online application tool containing 60mer oligonucleotides probes, which were designed based on the *Saccharina latissima* cDNA library. Prior to the microarray hybridizations total RNA labelled with the Agilent two-color low RNA Input Linear Amplification kit (Agilent Technologies, Waldbronn, Germany). The manufacturer's labelling protocol (Agilent) was slightly adjusted due the extensive length of 3`untranslated regions (UTRs) occurring in brown algae, cDNA synthesis was performed using a blend of T7 promoter primer and T7 nonamer primer used in equal molarity.

Total RNA from stress treatments was hybridized against the control treatment. All hybridizations were carried out in 4 biological replicates. Hybridization, microarray disassembly and wash procedure were performed according to manufacturer's instructions (Agilent).

Differential gene expression was analysed using the GeneSpring GX software platform version 11 (Agilent) with the implemented statistical tests. Statistical analyses included an ANOVA, followed by a post hoc test Tukey HSD with the Benjamini Hochberg FDR correction.

Statistical assessments of GO annotations, whose abundance is significantly different between the regulated genes within the various exposure treatments and the whole microarray, were performed with Blast2GO (Conesa *et al.*, 2005). Furthermore significantly enriched KEGG pathways were identified with KOBAS (http://kobas.cbi.pku.edu.cn/home.do) using a hypergeometric test ($p < 0.01$).

3. Summary of results

3.1 Characteristics of the EST library

In order to establish a comprehensive EST library under light and temperature stress, young sporophytes were exposed to different combinations of temperature, PAR, and UV radiation. Exposure experiments were conducted for 8 h, 24 h, and 2 weeks. RNA extraction of treated sporophytes yielded high amounts of RNA of good purity and quality. RNA from differently treated sporophytes was pooled for EST library construction (publication I).

454-Sequencing of the library resulted in 400,503 high quality ESTs with an average read length of 348 bp, which were assembled into 28,803 contigs. The average contig size was 963 bp, but sequences could be assembled into contigs of up to 7.4 kb. For 4218 (~15%) of the assembled contigs a significant BLAST match was found in the Swiss-Prot database. Comparison of the assembled ESTs against the NCBI non-redundant protein database yielded 8379 (~29%) sequences showing significant similarity to known proteins. Sequence mapping according to Gene Ontology (GO) resulted in functional classification of 4735 contigs (16%) in one or more GO categories, resulting in 27,843 GO terms in total. Interpro Scan led to the identification of 10,009 contigs (35%) with a recognizable protein motif. The most frequent protein domains found in the cDNA library were the protein kinase-like domain, serine/threonine-protein kinase-like domain, NAD(P)-binding and thioredoxin-like fold domain. Furthermore protein domains associated with protein kinase activity, including the serine/threonine protein kinase-like domain as well as calcium/calmodulin dependent protein kinase-like domain were highly abundant. Enzyme code (EC) annotation yielded in classification 480 contigs to one or more enzyme category numbers, providing a total of 625 ECs, which were mapped to 85 biochemical pathways. Figure 3 shows a mapping of all ECs to a global metabolism map from KEGG.

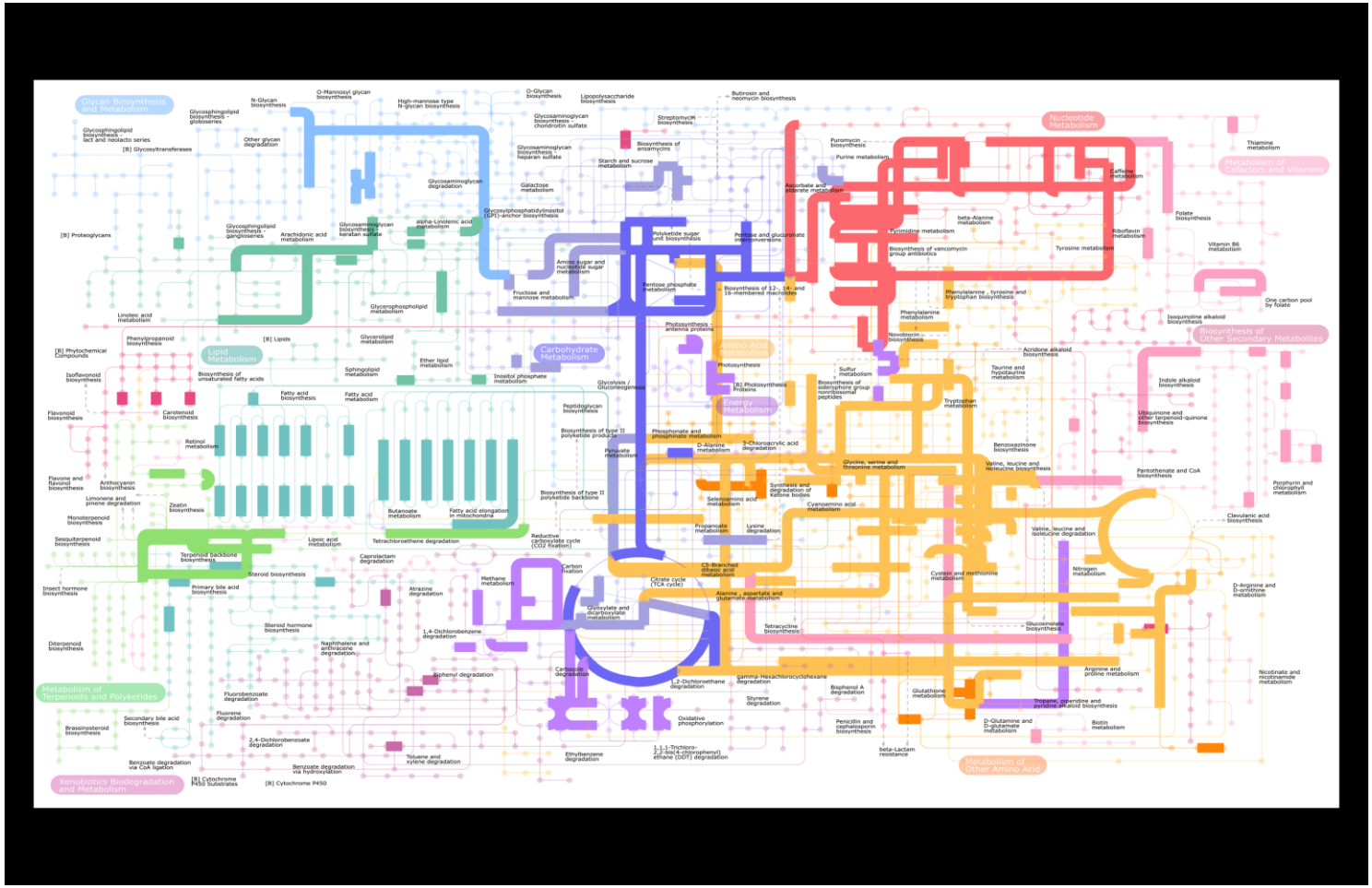


Figure 3. Global metabolism map from KEGG. Coloured lines represent enzyme reactions catalyzed by enzymes found in the EST library. Different colours correspond to different metabolism types (red: nucleotide metabolism, yellow: amino acid metabolism, dark blue: carbohydrate metabolism, lilac: energy metabolism).

Comparative genomics of *S. latissima* and *Ectocarpus siliculosus* indicated that the EST library exhibits genome coverage of approximately 70%, under the assumption of similar gene numbers in both species. GO term occurrence in *S. latissima* and *E. siliculosus* showed a similar distribution pattern among the root ontologies cellular component, molecular function and biological process. Analysis of specific protein domain abundances between the two algal species demonstrated that, the domains 'thioredoxin fold', 'thioredoxin-like fold', 'heat shock protein 70', and 'bromoperoxidase/chloroperoxidase C-terminal' are over-represented in the cDNA library. A plot of transcripts/gene-models per metabolic pathway involved in similar pathway maps from *S. latissima* and *E. siliculosus* revealed that the majority of EC counts of the two different species correspond to each other, resulting in a linear slope of ~ 0.81, indicating a high KEGG-pathway overlap between the cDNA library and the *E. siliculosus* genome (Fig. 4).

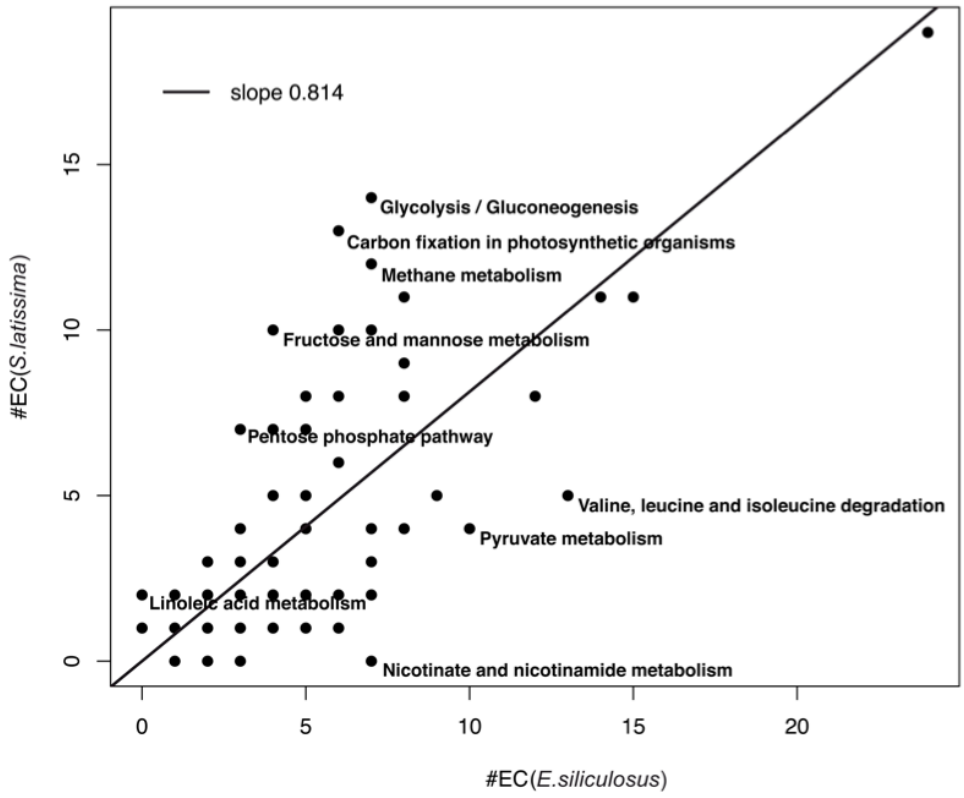


Figure 4. Comparison of transcript/gene-model counts from *S. latissima* and *E. siliculosus* in terms of ECs per metabolic pathway.

3.2 Short term acclimation to high PAR and temperature stress – physiological and molecular responses

In order to analyse molecular mechanisms underlying physiological acclimation to either high or low temperatures in combination with high light in *Saccharina latissima*, sporophytes were exposed for 24h to different combinations of light intensities and temperatures. For determining changes in gene expression patterns under different temperatures (2°C/17°C) and high photosynthetically active radiation (PAR), oligonucleotide microarrays covering 26,224 transcripts were employed (publication II). A multitude of transcriptional changes was observed: Altogether 42% of genes showed an altered expression after the exposure experiments in at least one stress treatment compared to the control treatment. More genes were differently up-regulated than down-regulated. Additionally, more genes were regulated at high than at low PAR conditions. The largest amount of transcriptional change was observed at high PAR and 17°C, when 8,334 genes (32%) were affected. The remaining three treatments (2°C & high/low PAR, 17°C low PAR) caused transcriptional changes for 13-19% (3,289 – 4,920) of the genes (Fig. 5).

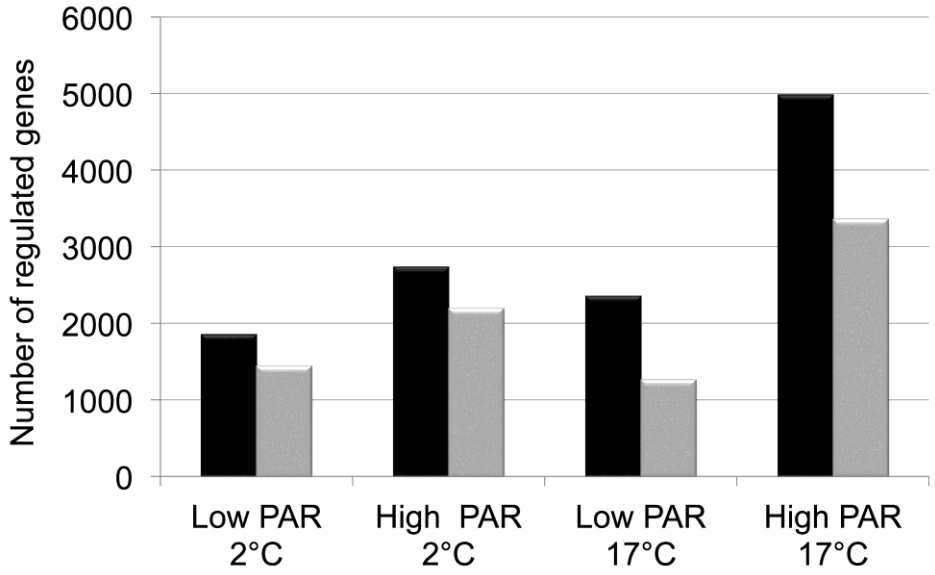


Figure 5. Numbers of differentially up- (black bars) and down-regulated (grey bars) genes after various stress treatments. Identification of regulated ESTs is based on microarray hybridizations and evaluated with an ANOVA against a control treatment with $n = 4$ and $p < 0.01$, followed by a post hoc Tukey test (HSD, $p < 0.01$).

For identifying an overlap of ESTs responsive to either high or low temperature within the different light treatments, a cross comparison was performed (Fig. 6). High temperature had stronger effects on gene expression in *S. latissima* than low temperature, and caused induction of 2,028 genes and repression of 988 transcripts. The effect of low temperature was less pronounced, in that 1,273 genes were found to be up-regulated and 1,002 genes down-regulated. A cross comparison revealed that the amount of high PAR responsive ESTs is dependent on temperature: at high PAR and low temperature 1,456 genes were induced whereas 1,188 genes were repressed; at high PAR and high temperature 2,949 genes were induced and 2,369 genes were repressed.

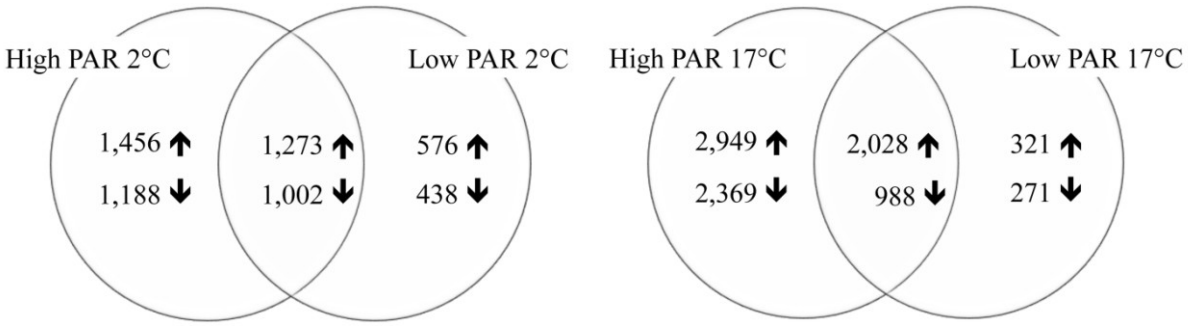


Figure 6. Venn diagram of responsive transcripts after 24h exposure to different light and temperature conditions. Numbers of responsive transcripts are separated in up (↑) and down (↓) regulated ESTs. The intersections display the number of ESTs regulated in both treatments.

3.2.1 Transcriptional regulation of metabolic processes

In general temperature had a smaller influence on metabolic processes than light. Two main temperature effects were observed, repression of transcripts encoding carbohydrate biosynthetic and catabolic processes at high temperature, and induction of genes associated with glycine, serine and threonine metabolism in response to low temperature.

A common feature observed after high PAR exposure was the up-regulation of catabolic processes for energy supply. The combination of high light and low temperature stress led to an induction of lipid catabolism and carbohydrate metabolism, furthermore to an increased number of induced gene activities in cellular amino acid biosynthesis, cellular nitrogen compound biosynthesis and nucleobase biosynthesis. Excessive light at high temperature was the most destructive stress condition for *S. latissima*, resulting in a strong repression of several metabolic processes, e.g. photosynthesis, carbohydrate metabolism and amino acid metabolism. A striking feature of the high PAR 17°C treatment was the significant induction of several genes involved in proteolysis, e.g. protease regulatory subunits and components of the ubiquitin-mediated proteolysis, which did not occur in any other of the stress treatments.

3.2.2 Photosynthesis and transcription of correlated genes

Sporophytes exposed to high photosynthetically active radiation conditions showed a significantly reduced maximum quantum yield of PS II (Fig. 7). The combination of high PAR and a temperature of 17°C resulted in the highest degree of photoinhibition, showing 90% reduced Fv/Fm as compared to the control after 24h of exposure. This was reflected by strong down-regulation (up to 60-fold) of transcripts encoding photosynthetic components, e.g. light harvesting complex proteins, photosystem II related proteins, porphyrin and chlorophyll metabolism proteins, and carbon fixation enzymes. High PAR at 2°C caused a decrease in maximum quantum yield between 40-50% compared to the control, on the transcriptional level up-regulation between 2-5 fold of photosystem II, thylakoid, and light harvesting complex protein correlated transcripts was observed.

The low PAR treatments by comparison induced no significant changes in maximum quantum yield of photosystem II, nevertheless light harvesting complex transcripts as well as some of the photosystem II transcripts were up-regulated at 2°C and down-regulated at 17°C with a fold change of about 2.

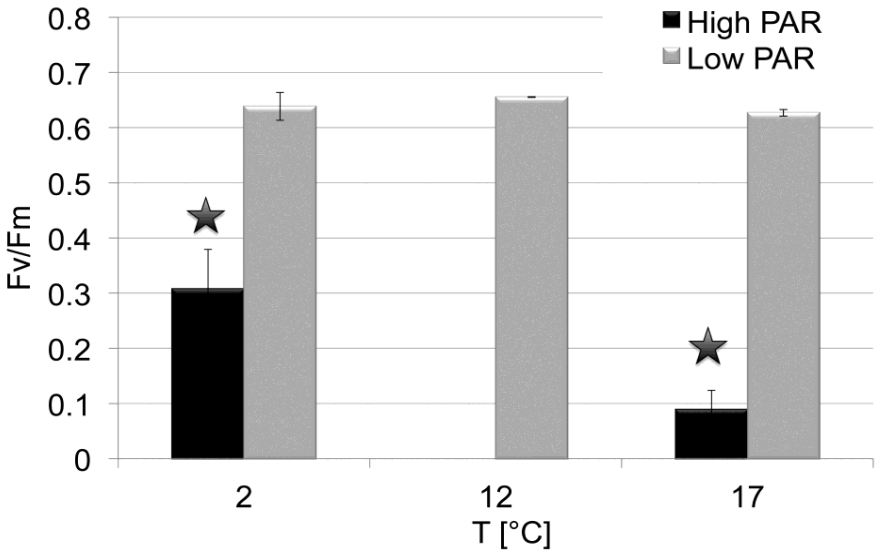


Figure 7. Maximum quantum yield of PS II (Fv/Fm) after 24h exposure to different temperature and radiation conditions. Standard deviations are represented by vertical bars (n=5). Asterisks mark significant differences in efficiency of PS II (two-way ANOVA with repeated measurements, $n = 5$, $p < 0.01$; post hoc Tukey test HSD, $p < 0.01$).

3.2.3 Induction of ROS scavengers and heat shock proteins

All ROS scavenging enzymes present in the library, except for superoxide and glutathione-S-transferase, showed the strongest induction after exposure to high light at 2°C. However, transcripts encoding for mitochondrial and chloroplastic superoxide dismutase were also induced at high temperatures. Significant induction of genes coding for enzymes of the ascorbate-glutathione cycle was detected, e.g. glutathione reductase (GR), which was up-regulated in all treatments except low PAR and 17°C. Additionally two genes of the chloroplastic thioredoxin system were detected, which were mainly up-regulated in response to high PAR

Differential induction of various heat shock proteins (Hsps) was also detected. Members of the Hsp 70 family were most strongly expressed after the high PAR 17°C treatment. Four transcripts, e.g. heat shock factor-binding protein, were solely up-regulated in response to high temperature in combination with high PAR. Interestingly three Hsps, Hsp 33, Hsp 60, and Hsp 90, respectively, were induced exclusively at low temperatures.

3.3 Short term acclimation to UVR and temperature stress – physiological and molecular responses

To investigate transcriptional changes underlying physiological short term acclimation to UV radiation at different temperatures, sporophytes of *S. latissima* were exposed for 24h to UV radiation at 2°C, 7°C and 12°C. Gene expression profiles were assessed by microarray hybridizations (publication IV).

UVR exposure led to a changed expression of 8,166 transcripts (32%) in at least one stress treatment compared to the control treatment. UVR at 2°C and 7°C had stronger effects on gene expression than UVR at 12°C, which was reflected by a higher number of regulated genes. Analysis of Gene Ontology terms showed, that UVR generally caused induction of transcripts associated with photosynthetic components, DNA replication, DNA repair, and vitamin B_6 biosynthesis. Following exposure to UVR at 2°C and 7°C, repression of transcripts correlated to carbohydrate metabolic processes, e.g. glucan metabolic process and polysaccharide metabolic process, was observed. KEGG pathway analyses indicated enhanced up-regulation of carotenoid and glutathione metabolism in response to UVR at 2°C. UVR at 7°C on the contrary caused enhanced repression of e.g. glycolysis, pentose-phosphate pathway and galactose metabolism.

3.3.1 Photosynthesis and transcription of correlated genes

Exposure to UVR resulted in all experiments in significant decreases in maximum quantum yield of PS II (Fv/Fm). UVR and temperature showed interactive effects, the degree of photoinhibition in response to the UVR treatment at 2°C was significantly higher than at 7°C and 12°C, respectively. However, highest overall number of regulated transcripts encoding for photosynthetic components was observed after the UVR treatment at 7°C.

Four genes associated with photosystem II and one gene correlated to photosystem I were detected among regulated transcripts. Transcripts coding for photosystem I reaction centre subunit II were induced only after exposure to UVR at 7°C, whereas photosystem II D2 protein was up-regulated in response to all UVR conditions, with expression changes between 2.3-fold and 2.5-fold. Furthermore, transcripts of the cytochrome B_6 complex were induced in response to all UVR experiments. Genes coding for light harvesting complex proteins were regulated contrary in response to UVR at 2°C and 7°C, fucoxanthin-chlorophyll a-c binding

protein was 20-fold induced, while light harvesting complex I 21 kDa was repressed. Induction of lumenal thylakoid proteins occurred after exposure to UVR at 7°C.

3.3.2 Induction of DNA replication and repair enzymes

UVR exposure caused induction of transcripts correlating to DNA replication and repair. UVR exposure at 2°C and 7°C had stronger effects than UVR at 12°C.

Several genes involved in DNA replication, e.g. DNA gyrase subunit b and DNA polymerase I, were induced after UVR at 2°C and 7°C, with a stronger induction in response to UVR at 7°C. Transcripts correlating to replication factor proteins, e.g. replication factor-a protein and replication protein-a 69 kDa DNA-binding, were up-regulated after all UVR treatments with expression changes between 2.9-fold and 3.7-fold.

Four genes encoding for DNA repair enzymes were also detected. Transcripts encoding deoxyribodipyrimidine photolyase family protein, rad51 DNA recombination repair protein and x-ray repair cross-complementing protein 6 were up-regulated in response to UVR at 2°C and 7°C. DNA repair protein uvh3 showed enhanced transcript abundance in all treatments. All of these transcripts, except rad51 DNA recombination repair protein, showed the highest transcript abundance after exposure to UVR at 2°C.

3.3.3 Induction of ROS scavengers

Five regulated genes encoding for reactive oxygen species (ROS) scavenging enzymes were identified. Induction between 3.6-fold and 5.1-fold of genes coding for l-ascorbate peroxidase and dehydroascorbate reductase was observed in response to UVR at 2°C and 7°C, and a higher transcript abundance was detected under UVR at 7°C. These treatments furthermore induced thioredoxin and thioredoxin reductase, the latter featuring expression changes between 8.9-fold and 9.5-fold. Chloroplastic alternative oxidase was up-regulated in response to all UVR treatments, with expression changes between 2.2-fold and 4.9-fold. Here, the strongest induction occurred following exposure to UVR at 2°C

3.4 Long term acclimation –physiological and molecular responses to high PAR, UVR and temperature

This study aims to analyse the molecular mechanisms of long-term acclimation to both high and low levels of photosynthetically active radiation, in combination with UVR at different temperature regimes. Sporophytes of *Saccharina latissima* were subjected for two weeks to different combinations of light conditions at three temperatures (2, 7 & 12°C), subsequently gene expression patterns were determined by microarray hybridization (publication III).

After the exposure experiments, 6,563 transcripts (26%) showed differential regulation patterns for at least one stress treatment compared to the control treatment (2°C/7°C/12°C & low PAR). The strongest effect on gene expression was observed in the 2°C high PAR + UVR treatment when 2,228 genes (9%) showed significant differential expression compared to the control, followed by the 12°C high PAR + UVR treatment and the 12°C high PAR condition, at which 2057 (8%) and 2022 (8%) genes, respectively, were effected. The lowest number of regulated genes was detected in response to the 7°C low PAR + UVR condition, when only 397 genes (2%) showed an altered expression (Fig. 8).

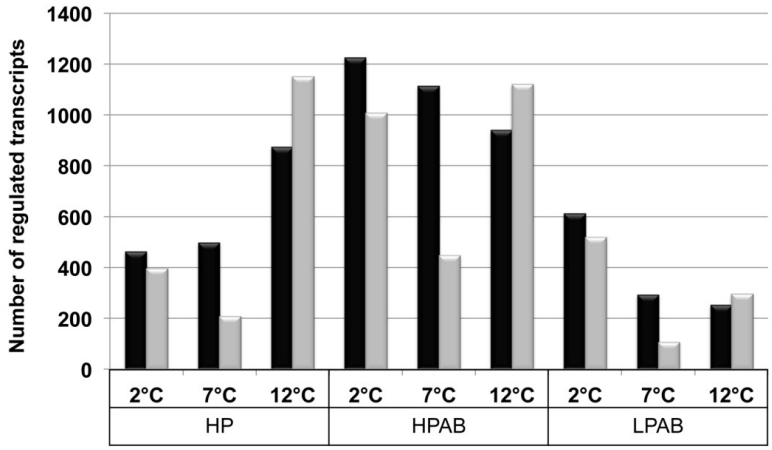


Figure 8. Total number of differentially up- (black bars) and down-regulated (grey bars) genes after exposure to 3 temperatures (2, 7 & 12°C) and 3 radiation conditions: low photosynthetically active radiation + UVR (LPAB), high photosynthetically active radiation (HP), and high photosynthetically active radiation + UVR (HPAB).

3.4.1 Transcriptional regulation of metabolic processes

Significantly enhanced abundance of transcripts associated with carotenoid metabolism was detected after exposure to low PAR + UVR at 2°C. Sporophytes subjected to high PAR + UVR at 2°C and 7°C showed enhanced induction of transcripts correlating to cellular amino acid metabolism, e.g. glycine, serine and threonine metabolism. These treatments furthermore caused induction of transcripts associated with porphyrin and chlorophyll metabolism. The 7°C PAR + UVR treatment indicated down-regulation of arachidonic acid metabolism and the PPAR signalling pathway. A striking feature was the observed strong up-regulation of transcripts correlating to vitamin B_6 metabolism under the combined stressors of high PAR and UV radiation.

3.4.2 Photosynthesis and transcription of correlated genes

Maximum quantum yield (Fv/Fm) remained stable at low photosynthetically active radiation (PAR) and under low PAR + UVR at all tested temperatures. Exposure to high PAR and high PAR + UV resulted in significant decreases in the maximum quantum yield of PS II, the highest degree of photoinhibition occurring after the high PAR + UV treatment. The rate of photoinhibition decreased due to acclimation over the course of the experiment; at the end of the experiment high PAR and high PAR + UV treatments featured average recovery rates of maximum quantum yield of 87% and 78%, respectively, compared to the low PAR and low PAR + UV treatments.

Several transcripts encoding photosynthetic components were up-regulated in response to the stress treatments. The highest number of responsive transcripts was found in the 2°C HPAB treatment, where all genes except for one, were found to be induced. The strongest up-regulation of up to 18-fold occurred for genes correlating to the light-harvesting complex, which were induced in the majority of treatments. All detected components of the photosystems, except the photosystem I p700 chlorophyll a apoprotein a1, were solely induced in response to the UVR treatments. Three transcripts encoding for photosystem I were detected, e.g. photosystem I reaction centre subunit II. These were primarily induced after the exposure treatments at 2°C. Transcripts related to photosystem II, such as photosystem II reaction centre protein y and photosystem II reaction centre protein D1/psbA, featured transcriptional changes between 2 – 2.8-fold in response to the UVR treatments.

3.4.3 Induction of ROS scavengers

Several transcripts encoding for antioxidative enzymes were significantly induced. Superoxide dismutase [Mn], ascorbate peroxidase, and thioredoxin h featured higher transcript abundance after exposure to high PAR and high PAR + UV conditions at 12°C. Superoxide dismutase [Fe] was induced in response to 2°C HP and 7°C/12°C HPAB. Chloroplastic thioredoxin, thioredoxin reductase and glutathione reductase were mainly up-regulated in response to the 2°C conditions and/or in response to high PAR + UV at 7°C.

3.5 Comparison of gene expression under UV radiation in field and culture grown *Saccharina latissima*

The purpose of this study was to explore whether sporophytes grown in the field would react to similar treatments in the same way as laboratory material, i.e. whether results obtained in laboratory experiments could be transferred to the behaviour in "real life". Such transfer would allow the prediction of behaviour of natural communities, e.g. under global change, based on laboratory experiments. Therefore, exposure experiments were conducted in Spitsbergen, Norway. *Saccharin latissima* sporophytes were collected by SCUBA diving, and after 2 days of acclimation subjected for 24h to UVR at three different temperatures (2,7 & 12°C) gene expression patterns were assessed via microarray hybridization. Afterwards, gene expression profiles of cultivated sporophytes and field sporophytes were compared.

Overall number of significantly regulated transcripts of cultured and field sporophytes in response to the exposure conditions indicated large differences (Fig. 9). In field material of *S. latissima* only 1,218 transcripts (5%) showed differential expression patterns under at least one stress treatment when compared to the control treatment (2,7,12°C & low PAR): among these the highest number of regulated transcripts (1014≈ 4%) was detected after the UV treatment at 2°C.

Exposure of cultured sporophytes on the contrary caused regulation of 8,166 transcripts (32%), strongest effect on gene expression was observed after UVR exposure at 7°C treatment. Furthermore strong interactive effects of temperature and UVR in cultured sporophytes were observed: at 12°C the amount of regulated genes was only half as much compared to the 2°C and 7°C treatment.

Genes, that were simultaneous regulated in cultivated and field sporophytes, exhibited differences in the level of expression fold change in response to similar stress conditions. However, principal effects of UVR were found to be similar in both cultured and field sporophytes. In general, exposure to UVR caused enhanced regulation of photosynthetic components, DNA repair and DNA replication as well as in vitamin B_6 biosynthesis.

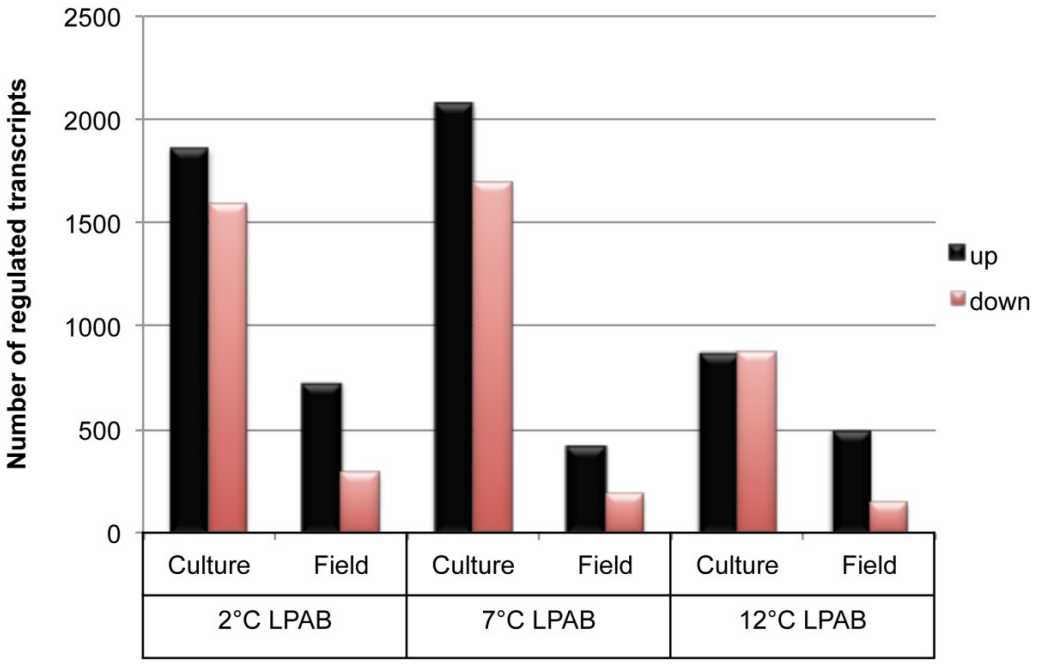


Figure 9. Number of significantly different up- (black bars) and down-regulated (red bars) genes in *Saccharina latissima* from culture and field after exposure to low photosynthetically radiation + UV (LPAB) at 3 temperatures (2, 7 & 12°C). Cultivated sporophytes were grown at 10°C, field sporophytes experienced growth temperatures between -3°C and 1°C.

3.5.1 Photosynthesis and transcription of correlated genes

The low PAR treatments induced no significant changes in maximum quantum yield of photosystem II (Fv/Fm). In contrast, UVR exposure experiments resulted in decreases in Fv/Fm. Interactive effects of UVR and temperature were observed in sporophytes of both origins, with a higher degree of photoinhibition occurring in response to the UVR treatment at 2°C than at 7°C and 12°C. No significant difference in the maximum quantum yield of PS II between cultured and field material, neither at the beginning nor after the exposure experiments was observed. Although the origin of sporophytes did not significantly influence the maximum quantum yield of photosystem (PS) II, it did lead to differences in the overall number of regulated genes encoding photosynthetic components, which was higher for the cultivated material. Furthermore, for simultaneously regulated genes in cultured and field material, differential expression changes were observed. Thylakoid proteins were up-regulated in a higher number of treatments in field sporophytes when compared to cultured

sporophytes. Photosystem I reaction center subunit II was induced following all UVR exposures in field sporophytes, however, only in response to one treatment in cultivated material. In addition, transcripts associated to photosystem II, e.g. photosystem II D2 protein, were generally stronger induced in field material.

3.5.2 Induction of DNA replication and repair enzymes

Induction of transcripts correlating to DNA replication and repair was observed in response to all UVR exposure experiments. As previously observed, higher numbers of regulated transcripts associated with DNA metabolism were detected in cultured sporophytes.

Most genes involved in DNA replication, e.g. DNA gyrase subunit b and DNA polymerase I, were induced after all UVR treatments except in cultivated sporophytes subjected to UVR at 12°C. Transcripts associated with the replication protein-a showed higher abundance after all UVR treatments. In contrast, the highest transcript abundance of replication protein-a in cultured sporophytes was observed after exposure to UVR at 2°C. For field sporophytes, the highest transcript abundance of replication protein-a was observed in response to UVR at 12°C. Detected genes encoding for DNA repair enzymes showed enhanced transcript abundance after all UVR treatments, except in cultured sporophytes under UVR at 12°C. Induction profiles of these transcripts were dependent on origin and temperature, but also on interaction of these two factors. X-ray repair cross-complementing protein 6 was generally stronger induced in field sporophytes than in cultivated sporophytes. Transcription of a deoxyribodipyrimidine photolyase family protein was highest in both sporophyte types after the 2°C UVR treatment, whereas DNA repair protein uvh3 showed highest transcript abundance in cultured material after the 2°C UVR treatment, in field material after the 12°C UVR condition.

3.5.3 Induction of ROS scavengers

Five transcripts coding for antioxidative enzymes were simultaneously regulated in both cultured and field sporophytes. We detected enhanced transcript abundance of e.g. l-ascorbate peroxidase, dehydroascorbate reductase and thioredoxin reductase after various UVR treatments. In general, UVR at 2°C and 7°C caused stronger induction in cultivated material when compared to field material. Contrarily, UVR at 12°C caused stronger up-regulation in field sporophytes as in cultured sporophytes.

4. Synopsis of discussion

4.1 EST library - a cost-effective genomic resource

The aim of this study was to create a *Saccharina latissima* cDNA library for future functional genomics studies on the mechanisms and pathways involved in temperature and light acclimation (publication I). 454-sequencing of the cDNA library yielded in 400,503 ESTs, which were assembled into 28,803 contigs with an average size of 963 bp. The average transcript length in *Ectocarpus siliculosus* is 2409 bp, and is composed of a 1563 bp coding region plus 845 bp of 3′ untranslated regions (Cock *et al.*, 2010). Hence, the number of 28,803 contigs is most likely an overestimate and does not reflect the actual number of genes in *S. latissima*. Non-overlapping fragments from the same gene will often not be assembled into one single contig, which can lead to redundancies.

BLAST sequence comparison showed similarities to known proteins for only 29% of the assembled ESTs. Brown algal EST libraries generally show low overall annotation rates of less than 50% (Wong *et al.*, 2007; Pearson *et al.*, 2010). One reason for this is the extensive length of 3′ UTRs occurring in brown algae (Apt *et al.*, 1995; Crépineau *et al.*, 2000; Cock *et al.*, 2010). Another reason for the low overall annotation rates is that the majority of available sequence data used for sequence comparison are from evolutionarily distant organisms. So far, only one brown algal genome (*E. siliculosus*, Cock *et al.*, 2010) and few chromalveolate genomes (*T. pseudonana*, Armbrust *et al.*, 2004; *P. tricornutum*, Bowler *et al.*, 2008) have been fully sequenced.

Frequent Interpro protein domains found in the cDNA library were the protein kinase-like domain, serine/threonine-protein kinase-like domain, NAD(P)-binding and thioredoxin-like fold domain. Protein kinases are involved in regulation of cell division, metabolism, and responses to external signals (Hrabak *et al.*, 2003). Serine/threonine-protein kinases catalyse the phosphorylation of serine and threonine residues in proteins, and play a prominent role in signal transduction (Edelman *et al.*, 1987). Cock *et al.* (2010) previously demonstrated that the evolution of multicellularity in brown algae is correlated with an increased number of signal transduction genes. Furthermore, analysis of predicted gene families gained by the *E. siliculosus* genome since divergence from the diatoms indicated a significant parallel gain in ontology terms correlated to protein kinase activities. This high abundance of protein kinase-domains in the cDNA library confirms these findings and highlights the importance of a

sophisticated signal transduction and processing system for multicellularity. Investigations on specific protein domain abundance between *S. latissima* and *E. siliculosus* revealed that the domains 'thioredoxin fold', 'thioredoxin-like fold', 'heat shock protein 70', and 'bromoperoxidase/chloroperoxidase C-terminal' were over-represented in the cDNA library. It was also shown that high light and high temperature leads to an increased expression of Hsps and genes with anti-oxidative function (Collén *et al.*, 2007). The enhanced appearance of these generally stress-associated domains indicates that the corresponding gene families are amplified in the *S. latissima* genome in comparison to that of *E. siliculosus*. The cDNA library also reflects the chosen stress conditions, and is therefore an excellent basis for further microarray investigations under relevant conditions.

Comparison of GO-term occurrence in *S. latissima* and *E. siliculosus* showed a similar distribution pattern of GO terms among the three root ontologies, i.e. biological process, molecular function and cellular component. Furthermore, comparative KEGG metabolic pathway mapping of transcripts/gene-models of *S. latissima* and *E. siliculosus* revealed that the majority of EC counts of the two different species correspond to each other, indicating a high KEGG-pathway overlap between the cDNA library and the *E. siliculosus* genome. Thus we conclude that the cDNA library is representative of the *S. latissima* transcriptome under the tested conditions and displays a rather complete gene catalogue of the species. Nevertheless, genes that are expressed under special conditions, such as darkness and reproduction, as well as specific genes expressed in other life stages of *S. latissima*, are most likely missing.

In total over 10,000 contigs were identified by BLASTx, Interpro protein motif annotation, or Gene Ontology, leading to functional genome coverage of approximately 70%. This study has confirmed the EST library as an efficient and cost-effective tool for gene discovery in non-model organisms. This large EST collection comprises an important resource for studies of gene expression, comparative RNA-seq mapping and annotation of the forthcoming genomic DNA-sequences of *S. latissima* and related kelp species.

4.2 Short- term acclimation to abiotic stress

4.2.1 Effects of high PAR and temperature stress

The present study (publication II) demonstrates that *Saccharina latissima* responds to high PAR and temperature stress with a multitude of transcriptional changes. The highest number of regulated transcripts was detected after exposure to high PAR at 17°C, whereas low PAR at 2°C caused the lowest number of regulated transcripts. High temperature had stronger effects on gene expression than low temperatures, indicating that high temperatures are more harmful for *S. latissima* than low temperatures, resulting in stronger efforts to compensate for the negative effects. Exposure to high temperature resulted in repression of e.g. carbohydrate biosynthetic and catabolic processes and transcripts correlated to photosynthesis and carbon fixation. On the one hand, this may be due to the increasing reaction rate of enzymes at elevated temperatures, which in many cellular processes tends to increase with a Q_{10} from about 2 (Weis, 1981). On the other hand, down-regulation of transcripts can partially be caused by energetic and mass limitations of the full transcriptome, favouring transcripts of acute and chronic stress response. Low temperatures caused the induction of transcripts associated with glycine, serine and threonine metabolism. So far, only a few studies have investigated amino acid metabolism of macroalgal species (Nagahisa *et al.*, 1995; Gravot *et al.*, 2010) and until now no data are available on the influence of cold acclimation on the amino acid metabolism of macroalgae. However, serine is involved in the final step of cysteine biosynthesis (Noji *et al.*, 1998). Cysteine, glycine and glutamate are essential for synthesis of Glutathione (GSH), a reducing co-factor for several enzymes involved in ROS detoxification (Noctor *et al.*, 1997; Noctor, 2006). Thus the induction of the glycine, serine and threonine metabolism in *S. latissima* at low temperature may reflect a higher demand of GSH due to an increase of photooxidative stress.

Light stress in combination with temperature stress caused interactive effects on the transcriptional level, the amount of high PAR responsive transcripts doubled at high temperature. General features of stress response in sporophytes subjected to high PAR were induction of catabolic processes for energy supply and antioxidant enzymes.

Stress in general results in an induction of genes with energy generating, heat shock and antioxidant functions, whereas growth related genes are mostly repressed (López-Maury *et al.*, 2008). So far relatively few studies have investigated gene expression under stress in macroalgae, but these have produced similar results, namely the repression of genes related to

growth and primary metabolism and induction of energy generation and production of protectants (Collén *et al.*, 2006; Collén *et al.*, 2007; Dittami *et al.*, 2009). Exposure to high PAR at low temperatures caused additionally up-regulation of e.g. nitrogen compound biosynthesis, amino acid biosynthesis and aminoacyl-tRNA metabolism. Taken together, these results suggest that energy was generated for removal and replacement of damaged proteins as well as for the synthesis of stress related genes, e.g. Hsps and ROS scavenging enzymes.

Excessive light at high temperature on the contrary resulted in a strong repression of several crucial metabolic processes, e.g. photosynthesis, carbohydrate metabolism and amino acid metabolism as well as to induction of several genes involved in proteolysis. These findings indicate that high PAR at 17°C was the most destructive stress condition for *S. latissima* and resulted in the highest degree of protein dysfunction.

Significant induction of several transcripts associated with reactive oxygen species (ROS) scavenging mechanisms was detected. Here, the highest transcript abundance occurred after exposure to high light at 2°C, indicating maximum rates of ROS formation under excessive light in combination with low temperature. These findings agree with previous studies that have demonstrated increased activity on ROS scavenging enzymes in macroalgae after exposure to several abiotic stresses, namely low temperature and UV radiation (Collén and Davison, 2001; Aguilera *et al.*, 2002; Burritt *et al.*, 2002; Shiu and Lee, 2005).

Various heat shock proteins (Hsps) were identified, which were differentially induced in response to the experimental conditions. Interestingly, some Hsps were exclusively induced at low temperatures, whereas others were solely up-regulated in response to high PAR at 17°C. This suggests that the sophisticated regulation of Hsps in *S. latissima* is a prominent part of acclimation, not only to temperature but also to combined environmental stresses such as high PAR in combination with high temperature.

Fluorescence measurements showed that high photosynthetically active radiation conditions caused a significant decrease in the maximum quantum yield of PS II, here the combination of high PAR and a temperature of 17°C resulted in the highest degree of photoinhibition. On the transcriptional level this was reflected by strong down-regulation (up to 60-fold) of transcripts responsible for photosynthetic components, e.g. encoding for light harvesting complex proteins and photosystem II related proteins. Fluorescence measurements and gene expression studies indicate a breakdown of photosynthesis, suggesting that *S.*

latissima from the Arctic is particularly susceptible to chronic photoinhibition at high PAR combined with high temperature conditions. High PAR at 2°C on the contrary caused induction between 2-5-fold of photosystem II, thylakoid, and light harvesting complex protein correlated transcripts

Specific transcriptional changes for those treatments causing decreased maximum quantum yield were induction of photosystem II D2 protein at high PAR 2°C, as well as strong repression of photosystem II reaction center protein D1/psbA at high PAR 17°C.
Excessive light causes photoinhibition, whereas chronic photoinhibition occurs when the rate of damage exceeds the capacity of the PSII repair and is associated with the inactivation and degradation of PS II reactions centre (Huner *et al.*, 1996; Franklin and Forster, 1997; Hanelt *et al.*, 1997; Adams *et al.*, 2004). The recovery mechanisms of photosystem II include removal of damaged D1 proteins and the subsequent replacement by newly synthesized molecules for the PSII (Nymark *et al.*, 2009). A recent study has demonstrated that photodamage of PS II is not only associated with damage within the PSII reaction center, but also with a decreased repair rate caused by inhibition of the synthesis of the D1 protein (Takahashi and Badger, 2011). The observed decrease in maximum quantum yield of PS II and the reduced transcript abundance of D1 thus suggest that the severe damage to PS caused by the 17°/high PAR treatment could not be overcome by transcriptional regulation in *S. latissima*. The observed effect of high PAR stress on the photosynthesis of *S. latissima* might be due to the low light adaption of the species. Low light adapted subtidal macroalgae are more sensitive to high PAR stress than high irradiance adapted macroalgae (Häder and Figueroa, 1997). It was furthermore demonstrated that in *S. latissim*a, photosynthetic efficiency decreases with increasing temperatures (Davison *et al.*, 1991). However, hyper-optimal temperatures of up to 22°C did not affect photoinhibition in *S. latissima* collected from the Atlantic coast of Maine and Long Island Sound (Bruhn and Gerard, 1996). These contradictory results may be due to ecotypic differentiation within *S. latissima,* helping to explain the huge capacity for climatic adaption and therefore its wide geographic distribution.

In conclusion this study gives insights into underlying molecular processes of acclimation to light and temperature stress in *S. latissima*. Molecular acclimation mechanisms include adjustment of the primary metabolism, induction of several ROS scavengers and a sophisticated regulation of Hsps. A temperature of 17 °C affected the gene expression in *S. latissima* stronger than a temperature of 2 °C, indicating that *S. latissima,* as a cold adapted species, requires a larger scale metabolic reorganization for acclimating to high temperatures,

than to low temperatures. Furthermore the combination of the stress factors light and temperature caused interactive effects on photosynthesis and gene expression profiles. Simultaneous influence of multiple stress factors can elevate their damaging effects, and can lead to an increased susceptibility to additional stresses (Alexieva *et al.*, 2003; Wernberg *et al.*, 2010). A possible consequence of this reduced resilience for kelps includes local extinctions of population near the southern distribution limit as well as a shift in its distribution towards the Arctic (Müller *et al.*, 2009).

4.2.2 Effects of UVR and temperature stress

Gene expression analysis of *S. latissima* subjected for 24h to UVR (publication IV) revealed that UVR at 2°C and 7°C affected gene expression stronger than UVR at 12°C, which was reflected by a higher number of regulated genes. Primary transcriptomic responses to UVR include enhanced regulation of photosynthetic components, DNA repair and DNA replication. Previous studies on UV stress in macroalgae have indicated that UVR negatively influences photosynthesis and causes DNA damage (Hanelt *et al.*, 1997; Bischof *et al.*, 1998; Dring *et al.*, 2001; Van De Poll *et al.*, 2001; Franklin *et al.*, 2003; Zacher *et al.*, 2009). The transcriptomic data obtained in this study support these findings, indicating photosynthesis and DNA to be prime targets of UVR in macroalgae. A striking feature was the observed induction of vitamin B_6 biosynthesis after all UVR treatments. Vitamin B_6 is a water-soluble vitamin, which exhibits a high antioxidant activity, and is therefore suggested to be involved in protection from oxidative stress (Ehrenshaft *et al.*, 1999; Mooney and Hellmann, 2010). In *Arabidopsis* vitamin B_6 has been demonstrated to be crucial for acclimation to osmotic and oxidative stress (Shi *et al.*, 2002; Chen and Xiong, 2005). Thus we suggest that vitamin B_6 might be involved acclimation to UVR in *S. latissima* and may potentially be participating in protection from oxidative stress.

Exposure to UVR caused significant decreases in photosynthetic efficiency. The degree of photoinhibition was in fact higher in response to the UVR treatment at 2°C than at 7°C and 12°C. These findings are consistent with previous investigations on interaction of UVR and temperature on photosynthesis in macroalgae. Studies on interactive effects of those two stressors in macroalgae has previously shown similar results: the kelp species *Alaria esculenta* featured higher maximum quantum yield of PS II under UVR 13°C and 17 °C compared to 4°C and 9°C (Fredersdorf *et al.*, 2009). Another study has also demonstrated that

the degree of photoinhibition in two investigated *Ulva* species was smaller at 10°C than at 0°C (Rautenberger and Bischof, 2006).

Several transcripts encoding for photosynthetic components were significantly regulated, the lowest number of regulated genes was detected after the UVR exposure at 12°C. Induction of transcripts associated to photosystem II, e.g. photosystem II D2 protein, was observed in response to all UVR conditions. In contrast, transcripts coding for photosystem I reaction center, e.g. subunit II were only induced following exposure to UVR at 7°C. The higher number of regulated transcripts, and the higher number of conditions featuring regulation of these transcripts indicate that photosystem II is more prone to UVR damage than photosystem I. These findings are consistent with former studies demonstrating that PS II is more sensitive to UVR than PS I (Franklin *et al.*, 2003; Vass *et al.*, 2005). Several studies on macroalgae have shown that UVR leads to degradation of several photosynthetic components; recovery mechanisms include degradation and biosynthesis of damaged photosynthetic reaction centre proteins (Franklin *et al.*, 2003; Bischof *et al.*, 2006). Repair mechanisms of UVR damaged PS II reaction centres in cyanobacteria are associated with increasing the transcript pool of photosynthetic reaction centre proteins (Campbell *et al.*, 1998; Huang *et al.*, 2002). Hence, the induction of transcripts encoding for PS II and PS I may reflect enhanced repair rates of photosynthetic proteins due to an increased protein turnover under UVR stress. Furthermore, fluorescence measurements and microarray analyses suggest a temperature optimum for photosynthesis of 12°C. The transcriptomic data particularly support previous studies which have demonstrated the optimum growth range for *S. latissima* being between 10°C and 15°C (Fortes and Lüning, 1980; Bolton and Lüning, 1982; Lüning, 1984).

Most transcripts associated with DNA replication, e.g. DNA gyrase subunit b and DNA polymerase I, were induced after all UVR treatments, except in sporophytes subjected to UVR at 12°C. Studies on the effect of UV radiation on gene expression in *Synechocystis sp* and *Arabidopsis thaliana* have revealed similar results, namely the up-regulation of genes coding for DNA replication (Huang *et al.*, 2002; Molinier *et al.*, 2005). Additionally, four genes encoding for DNA repair enzymes were detected, which indicates enhanced transcript abundance after all UVR treatments except in sporophytes subjected to UVR at 12°C. These transcripts were found to be associated with three different DNA repair processes: photoreactivation, homologous recombination, and nucleotide excision repair. These results suggest that experimental UVR caused severe DNA damage in sporophytes of *S. latissima*. It was shown that UVR induces oxidative damage to DNA, crosslinks between DNA-protein

and DNA-DNA, double-strand breaks of DNA, as well as enhances formation of cyclobutane-pyrimidine dimers (CPDs) (Hall *et al.*, 1992; Britt, 1999). Gene expression analysis indicates that UVR acclimation in *S. latissima* comprehends sophisticated regulation of different DNA repair processes. Furthermore a temperature of 12°C seems to ameliorate the negative effects of UVR on DNA.

4.3 Long-term acclimation to high PAR, temperature and UVR stress

In order to study long-term changes in gene expression in response to different combinations of photosynthetically active radiation, UVR, and temperature in *S. latissima* sporophytes were either subjected to low or high PAR in combination with UVR at three different temperatures for two weeks (publication III). The highest number of regulated genes occurred in response to high PAR + UV at 2°C, where 2,228 genes (9%) showed a significantly differential expression when compared to the control, suggesting that acclimation to this condition requires the highest amount of metabolic reorganization. Whereas combinations of low PAR and UVR caused mainly enhanced regulation of photosynthesis, high PAR + UVR induced regulation of several metabolic pathways, e.g. induction of glycine, serine and threonine metabolism at 2°C and 7°C. As previously mentioned, serine is involved in the final step of cysteine biosynthesis. Cysteine, glycine and glutamate are essential for synthesis of glutathione (GSH), a reducing co-factor for several enzymes involved in reactive oxygen species (ROS) detoxification (Noctor *et al.*, 1997; Noji *et al.*, 1998). The induction of transcripts from glycine, serine and threonine metabolism might be caused by an increased production of ROS during exposure to high PAR + UV at 2°C and 7°C.

Both UVR treatments at 2°C furthermore caused induction of transcripts associated with carotenoid metabolism. Carotenoids comprise important components of the light-harvesting complex where they possess a dual function in light harvesting and photo-protection as they scavenge singlet oxygen and trap triplet states of chlorophyll (Horton and Ruban, 2005; Standfuss *et al.*, 2005). Studies in cyanobacteria have indicated that carotenoids may be involved in the inactivation of UV-B induced radicals in photosynthetic membranes (Woodall *et al.*, 1997; Götz *et al.*, 1999). Hence the induction of the carotenoid metabolism may be connected on one hand to the observed enhanced synthesis of light harvesting complexes in these treatments, and on the other hand to enhanced appearance of UV-induced

radicals at 2°C. Interestingly, transcripts correlated to vitamin B_6 metabolism were strongly induced under the combined stressors high PAR and UV radiation. Vitamin B_6 is a water-soluble vitamin that acts as a cofactor for a large number of essential enzymes, mostly associated with amino acid synthesis (González *et al.*, 2007; Leuendorf *et al.*, 2010; Mooney and Hellmann, 2010). Due to its high antioxidant activity it is thought to play an important role in protecting cells from oxidative stress (Ehrenshaft *et al.*, 1999). Studies on *Arabidopsis* revealed that vitamin B_6 is linked to stress responses and that it is crucial for acclimation to oxidative and osmotic stress (Chen and Xiong, 2005), additionally it seems to function in photo-protection (Titiz *et al.*, 2006). The induction of vitamin B_6 metabolism seems to be a critical component of the molecular acclimation mechanisms to excessive light in *Saccharina latissima* and could enable preventing damage by oxidative stress.

Maximum quantum yield of PS II (Fv/Fm) remained stable at low PAR as well as under low PAR + UVR under all tested temperatures. In contrast, sporophytes exposed to high PAR and high PAR + UVR showed a significant decrease in maximum quantum yield of PS II, with the strongest photoinhibition occurring after the high PAR + UVR treatments. The finding of unaltered maximum quantum yield of PS II after exposure to UVR at low PAR conditions suggests that the observed photoinhibitory effects are mostly caused by high PAR. Previous studies demonstrated that photoinhibition in several macroalgae was mainly induced by white light (Hanelt *et al.*, 1997; Bischof *et al.*, 1999; Dring *et al.*, 2001).

Transcriptomic analyses revealed induction of several components of both photosystems**,** primarily after exposure to UVR at 2°C and 7°C. Photosystem II showed a higher susceptibility towards UV radiation than photosystem I, which was reflected by the higher number of regulated transcripts correlated to PS II, and by the higher number of treatments featuring induction. Similar results were obtained in previous studies, which showed that the PS II is the primary target of UV-B radiation (Huang *et al.*, 2002; Franklin *et al.*, 2003; Vass *et al.*, 2005). As previously mentioned, studies in cyanobacteria have demonstrated repair of UV damaged PS II reaction centre by functions by increasing the transcript pool. The accumulation of these transcripts subsequently leads to a gradual replacement of the damaged D1 reaction centre (Sass *et al.*, 1997; Campbell *et al.*, 1998; Máté *et al.*, 1998; Huang *et al.*, 2002). Gene expression profiles indicate that the molecular mechanisms responsible for the repair of UV-induced photodamage are similar to those discovered in cyanobacteria.

Two main expression patterns of the different transcripts involved in ROS scavenging

were observed as either induction at 2°C or 12°C. These patterns may be caused by the subcellular location of the encoded gene products and possibly reflect changes in ROS levels in chloroplasts and mitochondria. In green plants, key components of ROS scavenging mechanisms, such as ascorbate-glutathione cycle and the thioredoxin system, are localized in several cellular compartments, and are encoded by single organelle targeted isoforms (Mittova *et al.*, 2000; Chew *et al.*, 2003; Jaquot *et al.*, 2009). The induction of glutathione reductase, chloroplastic thioredoxin and thioredoxin reductase in response to high irradiance at 2°C might be correlated to enhanced photo-oxidative stress. Excessive light generates photo-oxidative stress through an over-reduction of the photosynthetic electron transport chain. Low temperature leads to an additional increase of photo-oxidative stress, partly by reducing the activity of the Calvin cycle (Pfannschmidt, 2003; Haghjou *et al.*, 2009). The observed induction of SOD [Mn] at 12°C suggests an increased ROS formation in mitochondria. Similar expression patterns of SOD [Mn], ascorbate peroxidase, and thioredoxin h indicate that all these transcripts are involved in mitochondrial ROS detoxification. Plant mitochondria have been overlooked for a long time as source of ROS, due to the fact that estimates suggest formation of mitochondrial ROS being considerably lesser than that of chloroplast and peroxisomes in light (Sweetlove *et al.*, 2002; Foyer and Noctor, 2003). However, subsequent studies have demonstrated that changes in mitochondrial ROS formation have negative consequences for the cell, e.g. damage and inhibition of mitochondrial proteins as well as peroxidation of the mitochondrial membrane (Rhoads *et al.*, 2006; Noctor *et al.*, 2007; Schwarzlander *et al.*, 2009). Taken together, the results of this study indicate the existence of compartment specific ROS scavenging mechanisms in *S. latissima.* Gene expression profiles of ROS scavengers furthermore suggest a high amount of photo-oxidative stress in response to the 2°C condition as well as enhanced mitochondrial ROS formation in response to excessive light at 12°C.

4.4 Comparison of gene expression in field and culture grown *Saccharina latissima*

It is crucial to consider to what extent laboratory studies may be used to predict environmental effects in the field. So far, only a few comparative studies of acclimation in laboratory and field grown macroalgae have been published (Bischof *et al.*, 1999; Sagert and Schubert, 2000). Hence, this study (publication IV) aims on investigating whether origin, and

therefore growth conditions of the algae, influences gene expression profiles under UVR and temperature stress.

Large differences in the number of regulated genes in cultured sporophytes and field sporophytes were observed, with higher numbers of regulated genes occurring in cultivated material of *S. latissima*. Furthermore strong interactive effects of temperature and UVR on gene expression in cultured sporophytes were observed, at 12°C the amount of regulated genes was only half as much compared to the 2°C and 7°C treatment. Field material in contrast did not exhibit such strong interactive effects of UVR and temperature in gene expression. Cultivated material exhibited a higher number of regulated genes, enriched GO terms, and over-represented KEGG pathways than field sporophytes, which indicates that cultured sporophytes must make stronger efforts of acclimating to UVR than field sporophytes. This could be caused by the different ages of the thalli (8 weeks versus 4 month for cultured sporophytes versus field sporophytes respectively). Previous studies revealed differences in UVR sensitivity in *S. latissima* with respect to the age of the thalli, which is partly due to age-dependent morpho-functional features (Dring *et al.*, 1996; Bischof *et al.*, 2002).

Additionally, genes simultaneously regulated in cultured and field sporophytes featured differences in the level of expression fold change in response to similar stress conditions. Field sporophytes were taken directly after the ice break-up, and should be therefore sensitive to UVR. However the results suggest, that field sporophytes are less sensitive to UVR than cultured sporophytes. For plants it was shown, that exposure to a single stress agent can lead to increased resistance to subsequent unfavourable impacts (Alexieva *et al.*, 2003). This might be also the case for field sporophytes of *S. latissima*, exposure to cold temperatures might led to an increased resistance to UV-B radition.

Nevertheless, observed general transcriptomic responses to UVR were similar in cultured and field sporophytes. These included regulation of photosynthetic components, DNA repair and DNA replication. UVR effects on macroalgae are described extensively. Studies on UV stress in macroalgae have shown that UVR negatively influences photosynthesis and causes DNA damage (reviewed by (Bischof *et al.*, 2006; Hanelt *et al.*, 2007; Karsten *et al.*, 2011). Analysis of transcriptional profiles supports these findings, indicating photosynthesis and DNA to be prime targets of UVR in macroalgae.

In all experiments, UVR led to a significant decrease in photosynthetic efficiency. Interactive effects of UVR and temperature were observed in sporophytes of both origins, the

highest degree of photoinhibition was caused by the UVR 2°C treatment, despite the different growth conditions of cultivated and field sporophytes. No interactive effects of origin and stress treatments on maximum quantum yield in *S. latissima* were detected, but differences in the overall number of regulated genes encoding photosynthetic components, being higher within the treatments conducted with cultured material. UVR exposure caused induction of transcripts correlating to DNA replication and DNA repair in all treatments. Once again, higher numbers of regulated transcripts and enriched GO terms were detected in cultured material. Interestingly, a temperature of 12°C seems to ameliorate the negative effects of UVR on DNA in cultured sporophytes, yet not in field sporophytes. These findings indicate a higher susceptibility of field material to UVR at high temperature compared to culture material. Most of the ROS scavenging enzymes were induced in field sporophytes of *S. latissima* subjected to UVR at 12°C, whereas only one up-regulated transcript was detected after this treatment in culture material. This could also be reflective of lower oxidative stress levels in culture material at 12°C.

In summary, principal effects of UVR, targeting mostly photosynthesis and DNA, were similar in culture and field sporophytes. This indicates that laboratory experiments are well suited to investigate basic molecular mechanisms of acclimation to abiotic stresses. Gene expression profiles indicated a higher susceptibility to UVR and a higher oxidative stress level at 12°C in field sporophytes compared to culture sporophytes. In contrast, cultured sporophytes must make stronger efforts to acclimate to UVR at 2°C than field sporophytes. These results clearly demonstrate the influence of growth conditions on transcriptomic underlying acclimation to stress. Cold acclimation of *S. latissima* from the field might have caused metabolic alterations to increase stress performance at low temperatures that simultaneously led to a higher susceptibility at 12°C. The interactive effect of UVR, temperature and origin underscore the importance of conducting multifactorial experiments with field material when predicting biological and environmental effects of changing abiotic factors in the field.

4.5 Conclusion

This study was performed to investigate the molecular mechanisms underlying acclimation to abiotic stress in *S. latissima*. The first aim was to establish a cDNA library for subsequent functional genomics studies on the mechanisms and pathways involved in stress acclimation.

The established cDNA library can be viewed as a relatively complete gene catalogue of the species (publication I). Comparative analysis with the genome of *E. siliculosus* revealed high functional genome coverage of 70% of the cDNA library. It therefore comprises an important resource for subsequent studies molecular studies in *S. latissima* and related kelp species.

Gene expression analysis demonstrated that *S. latissima* responds to abiotic stress with a multitude of transcriptional changes. Furthermore the combination of the stress factors high PAR, UVR and temperature caused interactive effects on photosynthesis and gene expression profiles, a finding which highlights the importance of multifactorial experiments. The results of this thesis support previous physiological studies as well as give new insights into transcriptional regulation underlying physiological acclimation. The detected regulation of various ROS scavenging enzymes (publication II, III, IV) seems to play a crucial role in acclimation to abiotic stress in *S. latissma*. This finding is supported by publication IV, which bears evidence for the existence of compartment specific ROS scavenging mechanisms. Furthermore, *S. latissima* exhibits a sophisticated regulation network of Hsps, which is involved in acclimation not only to temperature but also to combined environmental stresses such as high PAR in combination with high temperature (publication II).

The induction of glycine, serine and threonine metabolism plays an important role in acclimation to high irradiance at low temperatures, which may be due to higher demands of glutathione (GSH), a reducing co-factor for several enzymes involved in reactive oxygen species (ROS) detoxification (Publication II, III).

Acclimation to UVR includes enhanced regulation of photosynthetic components (publication III, IV). Transcriptional analysis indicated that photosystem II exhibits a higher susceptibility towards UV radiation than photosystem I. Furthermore, repair of UV damaged PS II reaction centre seems to functions by increasing the transcript pool for transcripts associated with PS II.

Short acclimation to UVR furthermore includes enhanced regulation of DNA replication and DNA repair (publication IV). Interestingly three different DNA repair

processes, photoreactivation, homologous recombination, and nucleotide excision repair were detected, indicating a sophisticated regulation of different DNA repair processes.

Another striking feature was the observed induction of vitamin B_6 biosynthesis after all UVR treatments, which seems to be a crucial component of UVR acclimation, and may participate in protection from oxidative stress (publication III, IV).

Comparative gene expression in field and cultured sporophytes demonstrated large differences in gene expression (publication IV). Nevertheless, general effects of UVR, targeting mostly photosynthesis and DNA, were similar in both cultured and field sporophytes. This indicates that laboratory experiments are well suited to investigate basic molecular mechanisms of acclimation to abiotic stresses. The higher induction rates of transcripts associated with ROS scavenging as well as DNA repair and replication indicate a higher sensitivity to UVR and a higher oxidative stress level at 12°C in field compared to cultivated sporophytes. At the same time cultivated sporophytes grown at 10 °C must make stronger efforts of acclimating to UVR at 2°C than field sporophytes, which is reflected by the finding that at 12°C the amount of regulated genes was only half as much compared to the 2°C and 7°C treatment in cultured sporophytes. These findings highlight the influence of growth conditions on transcriptomic regulation underlying acclimation to stress, and underline the importance of also conducting experiments with field material when aiming to predict effects of changing abiotic factors in the field.

4.6 Future perspectives

While it has been possible to provide several answers on the topic of molecular acclimation mechanisms in response to abiotic stress, it is clear that much additional work will be required before a complete understanding of this topic can be obtained. Although mRNA levels often indicate active metabolic patterns, in some cases it is not possible to extrapolate the enzymatic activity from the transcriptional level. Therefore, future studies should combine transcriptomic and proteomic approaches to gain a more detailed picture of the molecular processes of acclimation.

As different life history stages, e.g. gametophytes and spores, are more prone to abiotic stress, it is now important to conduct gene expression studies under combined stressors in gametophytes and spores of *S. latissima*. These results would give valuable information and would improve our knowledge on tolerance patterns to abiotic stress in *S. latissima*. Furthermore, this data could then be used to predict future shifts in geographical distribution patterns of this species due to global environmental changes.

With respect to the ecotypic differentiation occurring in *S. latissima*, similar studies on gene expression under abiotic stresses should be realised with *S. latissima* populations of the southern distribution boundary. Such studies could provide further insights into variability and acclimation potential among spatially separated populations.

In conclusion, it would be of great interest to investigate gene expression profiles in response to abiotic stress in other macroalgal species within the ecosystem kelp bed. These data would give valuable information on susceptibility of different species towards abiotic stress. Different stress responses might be subsequently correlated to vertical zonation patterns of those algae.

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Publications

Publication I

(in press)

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A comprehensive cDNA library of light- and temperature-stressed *Saccharina latissima* (Phaeophyceae)

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Macroalgae of the order Laminariales (kelps) are important marine coastal primary producers with prime significance for ecosystem function. Important factors influencing their distribution include light and temperature but the molecular basis of kelp responses to these factors is poorly understood. We therefore constructed a comprehensive cDNA library from RNA sampled under various light and temperature regimes, as a basis for future studies about the mechanisms and pathways involved in acclimation to light and temperature stress in *Saccharina latissima*. A total of 400 503 ESTs was assembled into 28 803 contigs. We were able to assign putative functions or orthology relationships to more than 10 000 contigs by BLASTx, Interpro protein-motif annotation, or Gene Ontology (GO). The most frequent Interpro protein domains found in the cDNA library were the protein kinase-like domain, serine/threonine-protein kinase-like domain, and NAD(P)-binding and thioredoxin-like fold domain. Enzyme code (EC) annotation yielded attributions for 480 contigs, providing a total of 625 ECs, which could be mapped to 85 biochemical pathways. Comparative genomics of *S. latissima* and *Ectocarpus siliculosus* indicated that our cDNA library gave a genome coverage of approximately 70%, assuming similar gene numbers in both species. GO term occurrence in *S. latissima* and *E. siliculosus* showed a similar distribution pattern among the root ontologies biological process, molecular function and cellular component. Comparative protein domain annotation of *S. latissima* und *E. siliculosus* showed that, probably due to the chosen stress conditions, the domains 'thioredoxin fold', 'thioredoxin-like fold', 'heat shock protein 70', and 'bromoperoxidase/chloroperoxidase C-terminal' are over-represented in the cDNA library.

Key words: cDNA library, EST, kelp, *Laminaria saccharina*, light acclimation, macroalgae, Phaeophyceae, *Saccharina latissima*, RNA extraction, temperature stress

Introduction

Kelps – large brown algae belonging to the order Laminariales – are distributed on rocky coastal shores in cold to temperate regions from the Arctic to the Antarctic (Lane *et al.*, 2006). They form huge beds, which are the primary habitats for many different invertebrates, e.g. bryozoans and crustaceans (Carlsen *et al.*, 2007). In temperate regions extensive kelp forests represent some of the most productive marine ecosystems (Phillips *et al.*, 2011). In addition to their prime importance for ecosystem function, several species of the Laminariales are economically important and used as a food source in Asia and Europe (Lane *et al.*, 2006), for the alginic acid food additive industry (Crépineau *et al.*, 2000), and as valuable sources of biochemical compounds and pharmaceuticals (Waaland *et al.*, 2004; Roeder *et al.*, 2005).

The vertical and latitudinal distribution of kelps is limited by their light and temperature demands (Wiencke *et al.*, 2006; Bartsch *et al.*, 2008). Therefore global warming could potentially have a significant impact on their performance and survival. Kelp species of the polar regions are subjected to strong seasonal changes in physical and chemical conditions, e.g. an extreme seasonality of light conditions (darkness or low light prevailing in winter and under ice cover, but 24-h light in midsummer), accompanied by low water temperatures (Kirst & Wiencke, 1995). The perennial kelp *Saccharina latissima* (formerly *Laminaria saccharina*) has a circumpolar distribution in the northern hemisphere and is common in polar to temperate coastal waters (Bolton *et al.*, 1983; Borum *et al.*, 2002). It grows in both clear and turbid coastal waters, from the intertidal down to 30 m depth, and is consequently exposed to a wide

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range of temperature and light conditions (Gerard, 1988). Growth in *S. latissima* is limited by light on a seasonal basis and light availability often determines the maximum depth for survival. Hence the ability to maintain a positive carbon balance under a variety of light conditions is important for the broad distribution of this species (Lüning, 1979, 1990). The presence of dense stands of *S. latissima* in polar regions suggests that this species possesses a high acclimatory potential to cold temperature and low light (Kirst & Wiencke, 1995; Borum *et al.*, 2002).

The basic ecophysiological requirements of *S. latissima* are well known. Many studies have been conducted on the growth and photosynthetic performance of *S. latissima* sporophytes under various abiotic conditions (Davison, 1987; Davison & Davison, 1987; Davison *et al.*, 1991; Bruhn & Gerard, 1996; Machalek *et al.*, 1996; Spurkland & Iken, 2011); other studies have focused on their reproduction (Lüning & Dring, 1972, 1975; Lüning, 1980, 1981*a*, 1981b). Recent research has been dominated by studies of the impact of increased UV radiation on photosynthesis and growth on different life stages of *S. latissima* (Apprill & Lesser, 2003; Wiencke *et al.*, 2004; Roleda *et al.*, 2006*a*, 2006*b*, 2007; Davison *et al.*, 2007).

In contrast, the molecular biology of Laminariales is poorly understood. Only a few studies have focused on genes or genomes from *Laminaria* species. Some of these dealt with specific genes or gene families e.g. vanadium-dependent haloperoxidases (Colin *et al.*, 2003, 2005), mannuronan-C5-epimerases (Nyvall *et al.*, 2003) and the light-harvesting antenna multigenic family (De Martino *et al.*, 2000). Only three cDNA approaches have been published: Crépineau *et al.* (2000) characterized 905 ESTs of the gametophytes and sporophytes of *Laminaria digitata*, Roeder *et al.* (2005) established a cDNA library of 1985 ESTs from *Laminaria digitata* protoplasts, and more recently a subtractive cDNA approach was used to examine oligoguluronate-induced transcriptional defence responses (Cosse *et al.*, 2009). Ideally, the whole genome of a *Laminaria* species should now be sequenced. This would certainly boost work on this important genus, judging by progress in understanding another brown alga, *Ectocarpus siliculosus* (Cock *et al.*, 2010). The availability of the *Ectocarpus* genome has stimulated several new studies, e.g. of the molecular basis of cell wall polysaccharide metabolism (Michel *et al.*, 2010), mannitol synthesis (Rousvoal *et al.*, 2011), developmental patterning (Le Bail *et al.*, 2011) and inter-strain variation (Dittami *et al.*, 2011). However, Phillips *et al.* (2011) have shown that kelps possess some of the largest genomes reported for brown algae. For example, *Saccharina latissima* has an estimated haploid genome size of 588–720 Mbp [contrast 214 Mbp for *E. siliculosus* (Cock *et al.*, 2010) and 125 Mbp for the angiosperm *Arabidopsis thaliana* (Kaul *et al.*, 2000)], making a genome project species costly and also challenging in terms of assembly and annotation. A more cost-effective genomic resource that can be developed for almost any organism is an expressed sequence tag (EST) library. This provides an entry point for gene and genome analysis, especially in non-model organisms that lack other sequence resources (Bouck & Vision, 2007). Furthermore, EST libraries can be used for gene discovery, genome annotation, microarray design and comparative genomics (Rudd, 2003).

Because of the ecological and economic importance of kelps, it is our long-term goal to understand the molecular basis of adaptation and acclimation of kelps to environmental changes, such as global warming and an increase in UV radiation. The aim of this study was therefore to create a cDNA library that would facilitate future genomic studies on the mechanisms and pathways involved in temperature and light acclimation. We generated ESTs from *S. latissima* under different light and temperature regimes and assembled them into 28 803 contigs, which were functionally annotated by GO-terms, protein motifs and KEGG pathway mapping. We also compared the *Saccharina latissima* dataset to the complete genomes from *Ectocarpus siliculosus* and the diatom *Phaeodactylum tricornutum*, in order to better understand common and diverse functions of the three species.

Materials and methods

Algal material

Saccharina latissima (Lane *et al.*, 2006) sporophytes were raised from gametophyte cultures, which were in turn established from the spores of fertile sporophytes collected by SCUBA diving in Kongsfjorden (79°N, 11°E; Svalbard, Norway). Male and female gametophytes were ground together using mortar and pestle, transferred to Petri dishes filled with Provasoli-enriched seawater (Starr & Zeikus, 1993) and cultured at 10 ± 1°C and 30 µmol photons m^{-2} s^{-1} photosynthetically active radiation (Biolux 36 W, Osram, Germany) with 18 h light : 6 h dark. After 2 weeks, developing sporophytes were transferred to aerated 5-litre culture bottles and grown with twice-weekly changes of enriched seawater until they reached a size of 5–7 cm. To avoid using endophyte-contaminated thalli for the exposure experiments, cultures were examined by microscopy: no evidence for endophytes was found.

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Irradiance and temperature experiments

Young sporophytes were exposed to six different radiation conditions and four temperatures (Table 1), resulting in 24 treatments. Samples were taken after 8 h, 24 h and 2 weeks). All experiments were conducted with five replicates. To distinguish the effects of different wavelengths, the experimental units were covered with filter foils permeable to wavelengths of: (1) 400–700 nm (filter foil URUV Ultraphan UV farblos, Difrega, Germany) for photosynthetically active radiation (PAR) treatments; (2) 320–700 nm (Folanorm SF-AS; Folex; Germany) for PAR + UVA treatments; and (3) 295–700 nm (URT 140 Ultraphan UV farblos, Difrega) for PAR + UVA + UVB treatments. PAR (20 or 100 µmol photons m^{-2} s^{-1}) was provided by Osram daylight fluorescent tubes (three or five tubes, for the 20 or 100 µmol photons m^{-2} s^{-1} treatments respectively: Biolux 36 W, Osram, Germany) and measured using a LI-250 light meter (LI-COR, Lincoln, USA). UV radiation was generated by three fluorescent tubes (UV A-340, 40 W; Q-Panel, USA) and measured with a Solar Light PMA-2100 (Solar Light; PA, USA). After the exposure experiments, the sporophytes were cleaned by rinsing and wiping the thalli with ethanol to remove epiphytes and subsequently frozen in liquid nitrogen and stored at –80 °C until further use. In total 360 samples were taken, differing in treatments and times of exposure.

RNA extraction

Frozen sporophytes were ground in liquid nitrogen and transferred to 2.0 ml Eppendorf tubes. 1 ml of extraction buffer (2% CTAB, 1 M NaCl, 100 mM Tris pH 8, 50 mM EDTA, pH 8) and 20 µl DTT 2M were added and mixed well. The mixture was incubated at 45°C for 10 min. One volume of chloroform : isoamylalcohol (24 : 1) was added, mixed vigorously for 10 min, and then centrifuged for 20 min at 20°C and 12 000 × *g*. 750 µl of the aqueous phase were transferred into a new tube and 0.3 volumes of 100% ethanol added and mixed gently by inverting the tube. One volume of chloroform : isoamylalcohol (24 : 1) was added and a second chloroform extraction followed. 500 µl of the supernatant were transferred to a new cup and total RNA extracted by using a Qiagen Plant Mini Kit (Qiagen, Hildesheim, Germany) according to the manufacturer's protocol for RNA extraction, including on-column DNA-digestion. The quantity and purity of the extracted RNA were determined by analysing the absorbance of the samples at 230, 260 and 280 nm, using a NanoDrop ND-100 spectrometer (PeqLab, Erlangen, Germany). An integrity check of the total RNA was performed with the RNA Nano Chip Assay (Agilent Technologies, Böblingen, Germany).

Table 1. Experimental light and temperature conditions used for exposure experiments.

	Irradiance			Temperature
	PAR (µmol m^{-2} s^{-1})	UV-A [W m^{-2}]	UV-B [W m^{-2}]	(°C)
Desk 1	23.8 ± 3.08	9.83 ± 1.17	0.42 ± 0.03	2, 7, 12, 17
Desk 2	107.77 ± 4.96	9.68 ± 1.2	0.43 ± 0.03	2, 7, 12, 17

cDNA library construction

RNA from differently treated sporophytes was pooled for cDNA library construction. From the total RNA, poly $(A)^+$ RNA was prepared, thus also minimizing potential bacterial contamination. The first strand synthesis was primed with an N6 randomized primer. Normalization was carried out by one cycle of denaturation and reassociation of the cDNA, resulting in N1-cDNA. Reassociated ds-cDNA was separated from the remaining ss-cDNA (normalized cDNA) by passing the mixture over a hydroxylapatite column. After hydroxylapatite chromatography, the ss-cDNA was double-stranded and amplified with 10 PCR cycles. For 454 sequencing (reviewed by Mardis, 2008) the cDNA in the size range of 500–700 bp was eluted from a preparative agarose gel.

Sequencing and assembly

The double-stranded cDNA was sheared and ligated to 454/FLX specific adaptors. This sequencing library was then processed according to the manufacturer's protocols (Roche) and sequenced on a 454–Roche next-generation sequencing machine. Assembly of the raw reads was done using the in-built Newbler assembler with the default values. The drawback of this assembler (and others) is that alternative 3′ and 5′ ends tend to be under-represented in the final contig data. Original sequencing files were uploaded at the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra; accession number SRR305166).

Functional annotation

Assembled ESTs, i.e. the contigs, were compared against the Swiss-Prot protein knowledgebase (http://www.uniprot.org/) (release 2010_7) and the NCBI non-redundant protein database (http://blast.ncbi.nlm.nih.gov/Blast.cgi, NCBI-nr), using the BLASTX algorithm with an E-value cut-off of $10e^{-7}$.

Mapping of the sequences according to Gene Ontology (GO) was done using the Blast2GO analysis tool (Conesa *et al.*, 2005). For a comparison of GO annotations derived from different sources, two GO mapping steps were performed, one by using the BLAST hits derived from NCBI and one based on the BLAST hits from Swiss-Prot. GO terms were categorized with the CateGOrizer (Hu Zhi-Liang, 2008) using the GO roots and GO Slim2 classification files. Enzyme code (EC) annotation was conducted through the direct GO-EC mapping file (http://www.geneontology.org/external2go/ec2go) with the annotation parameters: e-value-hit-filter of $1.0e^{-6}$, annotation cut-off of 55, GO weight of 5, and HSP-Hit coverage

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cut-off of 40. For functional annotations of protein domains, all contigs were searched by InterProScan, which includes the databases BlastProDom, FPrintScan, HMMPIR, HMMPfam, HMMSmart, HMMTigr, ProfileScan, ScanRegExp and SuperFamily (Quevillon *et al.*, 2005) (http://www.ebi.ac.uk/interpro/index.html). GO terms as mapped from InterPro hits were merged with the GO annotation from BLAST. Metabolic pathways analysing based on the ECs derived from Uniprot BLAST hits was done by using the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/kegg2.html).

Comparative genomics

The *Saccharina latissima* transcribed dataset was compared by BLAST with protein sets from *Ectocarpus siliculosus* and *Phaeodactylum tricornutum*. A threshold score value of 150 was used to reduce false positive hits. For each species pair the number of hits was then counted. Differentially expanded gene families yield hits according to their numbers and thus the matches between genomes differ slightly depending on the genome used as a query.

For a detailed characterization of the cDNA library of *S. latissima* we compared the cDNAs with the complete predicted protein set of *E. siliculosus*. To reduce errors due to different annotation approaches, we reannotated the genome of *E. siliculosus* by using the Blast2GO program (Conesa *et al.*, 2005). Sequences were blasted against the NCBI non-redundant protein database (http://blast.ncbi.nlm.nih.gov/Blast.cgi, NCBI-nr) and the Swiss-Prot protein knowledgebase (http://www.uniprot.org) (release 2010_7) using the BLASTp algorithm with an e-value cut-off of $10\,e^{-7}$. GO mappings were derived from NCBI as well as Swiss-Prot Blast hits. EC annotation was performed using the same annotation parameters as for *S. latissima*. Additionally, sequences were mapped according to domain or motif by InterProScan and newly acquired GO terms were added to the GO annotations. ECs from the Uniprot BLAST were used for KEGG metabolic pathway mapping (KEGG, http://www.genome.jp/kegg/kegg2.html).

For comparative analysis of functional coverage between the cDNA library and the published genome of *E. siliculosus*, different plots were created. We wished to know whether there is a general difference in specific domain abundances between the two algal species and therefore the coverage of protein domains of the library in the genome of *E. siliculosus* was estimated from a linear fit of hit-counts to Interpro-domains. The log–log plot was produced in R (http://www.r-project.org), showing a linear fit with slope 0.25. Prominent domains were marked manually in all plots. Comparison of KEGG-pathway coverage between the cDNA library and the *E. siliculosus* genome was given in a corresponding plot from Blast2GO EC annotation counts per metabolic pathway. A linear fit was obtained with slope 0.8. Visually over- and under-represented pathways were marked manually.

Results

RNA extraction

A novel protocol had to be established for RNA extraction because standard procedures proved inapplicable to brown alga, due to the high amounts of polysaccharides (Wang *et al.*, 2005; Varela-Alvarez *et al.*, 2006) and phenolic compounds (Lane *et al.*, 2006; Pearson *et al.*, 2006). The new method (see Materials and methods) yielded $16.53 \pm 4.99\,\mu g$ RNA per 250 mg of frozen tissue and the extracted RNA was of high purity, showing $A_{260/280}$ ratios of 2.17 ± 0.04 and $A_{260/230}$ ratios of 2.23 ± 0.22 (values are means $\pm$ SD, $n = 240$). Analysis of total RNA integrity demonstrated that each RNA extract was of high quality and featured distinct 28S and 18S rRNA bands (Fig. S1). The RNA was tested successfully in downstream applications, e.g. cDNA synthesis and microarray hybridizations (data not shown). We also applied this method successfully to other brown algae (e.g. *Laminaria solidungula*, *Alaria esculenta* and *Fucus serratus*, not shown) and so it may be the RNA extraction method of choice for the class Phaeophyceae.

Characteristics of the cDNA library

Sequencing resulted in 400 503 high-quality ESTs with an average read length of 348 bp. These were assembled into 28 803 contigs, which were used for further analysis, leaving 67 976 singletons comprising short and low quality reads (Table S1), which were discarded. The average contig size was 963 bp, but sequences could be assembled into contigs of up to 7.4 kb (contig 27856, 7407 bp). More than 14 430 (51%) of the clusters consisted of two to five reads, whereas only 235 (0.8%) clusters were composed of more than 100 reads. The length distribution of EST-assembled contigs is shown in Fig. S2. Singletons were not taken into account, since without a genome sequence we were not able to distinguish between true transcribed sequences and sequences prevented from assembly due to sequencing errors.

Five of the most abundant transcripts found in the cDNA library (Table S2) corresponded to ribosomal proteins, three transcripts were detected to be involved in each of the categories 'cellular process' (elongation factor tu, endonuclease-reverse transcriptase, DNA-directed RNA beta subunit) and 'carbohydrate metabolism' (e.g. carbohydrate binding family 6, mannuronan c-5-epimerase), and two for each of the categories 'transport' (pre-protein translocase subunit, inner membrane transport protein) and 'photosynthesis' (ATP synthase cf1 beta chain, photosystem I reaction centre subunit II).

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32%) and 'intracellular' (GO:0005622, 26%) were the most abundant cellular component categories. The NCBI BLAST hits for GO mapping yielded 5128 contigs with GO terms, with a total of 11 116 GO terms and a mean GO level of 5.2 ± 1.88. The distribution of GO terms among the different categories showed a similar distribution pattern as the Swiss-Prot derived GO terms, with slightly different percentages (Fig. S4).

Interpro. Functional annotation of protein domains resulted in 10 009 contigs containing a recognizable protein motif. The 15 most frequent Interpro protein domains are presented in Table S4. Within these, four protein domains (IPR011009, IPR017442, IPR008271, IPR020636) relate to protein kinases, whereas five correspond to WD40 repeats (IPR019781, IPR015943, IPR011046, IPR017986, IPR019782). The most common protein domains found in the cDNA library were the protein kinase-like domain (IPR011009, 103x), serine/threonine-protein kinase-like domain (IPR011009, 75x), NAD(P)-binding domain (IPR016040, 68x) and thioredoxin-like fold (IPR012336, 66x).

Metabolic pathway analysis. The KEGG metabolic pathway analysis assigned 480 contigs to one or more enzyme category (EC) numbers, providing a total of 625 ECs. The EC numbers were mapped to 85 biochemical pathways (Table S5). Of these, 82 pathways belonged to the class 'metabolism'; the classes 'genetic information processing', 'environmental information processing' and 'organismal systems' were represented by one pathway each. The 25 pathways with the highest numbers of member proteins are shown in Table S6. Classification of metabolic pathways into pathway categories revealed that 358 contigs were involved in basic metabolic pathways, including energy metabolism (99 contigs), carbohydrate metabolism (95 contigs), amino acid metabolism (67 contigs), nucleotide metabolism (65 contigs) and lipid metabolism (30 contigs).

Reannotation of the Ectocarpus siliculosus genome

In order to allow functional annotations from *E. siliculosus* to be compared with those from *S. latissima*, the *E. siliculosus* genome was reannotated using the same procedures as for *S. latissima*. A comprehensive comparison is shown in Table S3. Not surprisingly, the *E. siliculosus* protein set, derived from a complete genome, presents more features in every category than the more fragmented EST set of *S. latissima*.

Comparative genomics

A Venn diagram (Fig. 2) displays the extent to which sets of transcripts/gene models are unique or overlap between our cDNA library and the genomes of *E. siliculosus* and *P. tricornutum*. Differentially expanded gene families yield hits according to their numbers and thus the matches between genomes differ slightly, depending on which genome is used as the query. tBLAST analyses of the 28 803 *S. latissima* contigs resulted in 7611 sequences shared with one or both other genomes. Of these, 4672 contigs were similar to *E. siliculosus* sequences, 2901 contigs matched proteins from both *E. siliculosus* and *P. tricornutum*,

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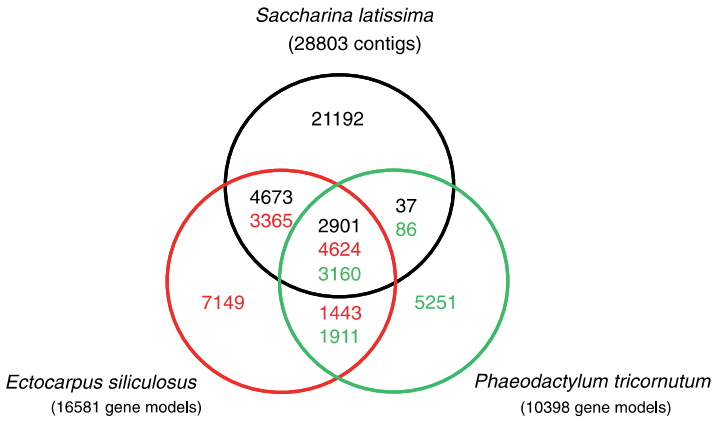

Fig. 2. Venn diagram illustrating the shared/unique sequences in *Saccharina latissima, Ectocarpus siliculosus* and *Phaeodactylum tricornutum*. BLAST analysis for each species dataset was performed against the other two species' datasets. Due to differential gene family expansions, different match counts in the bi-directional comparisons occur. Thus, match counts are depicted in the respective colour of the species (green for *P. tricornutum*, red for *E. siliculosus*, and black for *S. latissima*) used as query for the BLAST analysis. The high number of *S. latissima* specific contigs is due to the fragmented and partly non-coding nature of this EST dataset.

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nucleus–cytoplasm mRNA transport, subcellular localization and translation efficiency (Decker & Parker, 1995; Mignone *et al.*, 2002; Pesole *et al.*, 2002). However, until now no studies were conducted on the correlation of 3′ UTR length and function in brown algae. Another reason for the low overall annotation rates is the low coverage of chromalveolate sequences in public databases. Up to now only one brown algal species (*E. siliculosus*, Cock *et al.*, 2010) and few chromalveolates (*T. pseudonana*, Armbrust *et al.*, 2004; *P. tricornutum*, Bowler *et al.*, 2008) have been fully sequenced, and therefore the majority of the available sequence data used for sequence comparison are from evolutionarily distant organisms.

Gene Ontology is a controlled vocabulary used to characterize gene function annotation and classification; it was designed to standardize the representation of gene and gene product attributes across species and databases (Ashburner *et al.*, 2000). Consistent descriptions of gene products are essential for unambiguous comparisons of sequence data from different organisms (Cai *et al.*, 2006). The cDNA library from *S. latissima* displays a high functional coverage when compared with the gene models in the genomic sequences from *E. siliculosus*. Our Gene Ontology analysis yielded 33 304 GO terms in the genome of *E. siliculosus* and 27 843 GO annotations for the cDNA library from *S. latissima*, i.e. 83% of the total genome from *E. siliculosus*. Comparison of GO-term occurrence in *S. latissima* and *E. siliculosus* showed a similar distribution pattern of GO terms among the three root ontologies, i.e. biological process, molecular function and cellular component. Thus we conclude that our cDNA library is at least representative of the *S. latissima* transcriptome under the tested conditions and can be viewed as a rather complete gene catalogue of the species. However, genes expressed under special conditions such as sporogenesis, reproduction and darkness, and specific genes expressed in other life stages of *S. latissima*, e.g. gametophytes, are probably missing.

We assigned gene models from *E. siliculosus* and ESTs from *S. latissima* to EC numbers. For *E. siliculosus* 542 gene models could be linked to 707 ECs, which were mapped to 97 pathways. 480 sequences from *S. latissima* could be assigned to 625 ECs representing 83 KEGG pathways, i.e. 89%, 88% and 86%, respectively, of the numbers for the whole genome. The low EC annotation rate of the cDNA library and the genome of *E. siliculosus* shows the general limitations of automatic functional prediction of enzymes in terms of EC numbers based on sequence similarity. This agrees with a study by Devos & Valencia (2000) on the theoretical and practical basis of assigning potential protein functions on the basis of sequence similarity, where they observed a considerable number of cases in which a possible enzyme was not assigned to the corresponding EC number after an EC classification run. However, we were able to gain information about the KEGG pathway coverage of *S. latissima* compared with the genome of *E. siliculosus* and again the EST dataset seems to represent a significant part of the whole genome. A plot of transcripts/gene models per metabolic pathway of *S. latissima* and *E. siliculosus* involved in similar pathway maps showed that the vast majority of ECs correspond to each other in the two species, showing a linear slope of 0.81. We found a few pathways, including glycolysis/gluconeogenesis, carbon fixation in photosynthetic organisms, fructose and mannose metabolism, as well as methane metabolism, to be over-represented in the cDNA library of *S. latissima*. It is noteworthy that the enzymes found for methane metabolism in our cDNA library are mostly correlated to the production of halomethanes. Brown algae possess a metabolism for producing volatile halogenated organic compounds (VHOCs) like dibromomethane, dibromochloromethane and chloroiodomethane, which are thought to be involved in defence against microorganisms and ROS detoxification (Laturnus, 1996; Goodwin *et al.*, 1997; La Barre *et al.*, 2010). The categories valine, leucine and isoleucine degradation, pyruvate metabolism and nicotinate and nicotinamide metabolism were over-represented in *E. siliculosus*. The occurrence of either over- or under-represented pathways in either species might be due to the fact that the cDNA library of *S. latissima* is lacking genes that are not highly expressed under the chosen experimental conditions or due to the limitations of assigning protein functions based on sequence similarity.

Interpro protein motif database search yielded 11 915 protein domain annotations for *E. siliculosus* and 10 009 for *S. latissima* (84% of the former). Analysis of the protein motifs found in *S. latissima* ESTs showed that the majority of protein domains are associated with the term WD 40 repeat domain. WD 40 repeat proteins belong to a large conservative protein family and are important key structural signatures in a large number of cellular regulators involved in signal transduction, transcriptional regulation and cell cycle control (Holm *et al.*, 2001; Li & Roberts, 2001). Protein domains associated with protein kinase activity, including the serine/threonine protein kinase-like domain as well as the calcium/calmodulin dependent protein kinase-like domain, are also highly abundant. Protein kinases have several functions including regulation of cell division, metabolism, and responses to external signals (Hrabak *et al.*, 2003). Serine/threonine kinases can be divided into

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several families including the calcium-dependent protein kinase (CDPK) subfamily and the mitogen-activated protein (MAP) kinase subfamily (Hardie, 1999). In green plants calcium signalling has been shown to be involved in cell division and responses to hormones, light, pathogen elicitors and abiotic stresses (Charpenteau *et al.*, 2004). Mitogen-activated protein (MAP) kinases are serine/threonine kinases found in all eukaryotic cells (Jiménez *et al.*, 2004) and they are activated by environmental stimuli such as cold shock, wounding and pathogen infection (Hardie, 1999). Wheeler *et al.* (2008) proposed that the evolution of multicellularity requires the development of complex signalling mechanisms to regulate intercellular interactions e.g. in tissue differentiation and pathogen defence. It has recently been shown that the evolution of multicellularity in brown algae is correlated with an increased number of signal transduction genes, and analysis of predicted gene families gained by the *E. siliculosus* genome since divergence from the diatoms has indicated a significant gain in ontology terms associated with protein kinase activities (Cock *et al.*, 2010). Our results from the analysis of the *S. latissima* ESTs confirm these findings and highlight the importance of a sophisticated signal transduction and processing system for multicellularity.

We also found four over-represented Interpro domains in *S. latissima* (compared with *E. siliculosus*), i.e. thioredoxin fold, thioredoxin-like fold, heat shock protein 70 and the bromoperoxidase/chloroperoxidase C-terminal. Thioredoxins (Trx) are small, multifunctional proteins with a redox-active disulphide group, which are widely distributed among organisms. In plant tissues, two types of Trx systems have been described, the chloroplast-located ferredoxin Trx system and the cytosolic NADP Trx system (Balmer *et al.*, 2004). In chloroplasts, ferredoxin is reduced by light-driven photosynthetic electron transport chain, and serves as the electron donor for ferredoxin–thioredoxin reductase, which in turn reduces chloroplast Trx; reduced Trx interact with enzymes, e.g. of carbohydrate metabolism, and regulate their activity (Schürmann & Jacquot, 2000). Furthermore chloroplast Trx are critical for signalling via thioredox control (Arner & Holmgren, 2000). Cytosolic Trx of photosynthetic cells are reduced with electrons from NADPH via the NADP/thioredoxin system. A few functions have been proposed for the cytosolic Trx system in plants, e.g. mobilization of protein reserves during seed germination or hydrogen peroxide resistance (Schürmann & Jacquot, 2000).

Heat-shock proteins (Hsps) are molecular chaperones and involved in the folding, assembly, translocation and degradation of proteins. They play a crucial role in response to stress by re-establishing the original protein conformation, thus helping to achieve cellular homeostasis (Wang *et al.*, 2004). They are induced by a wide range of stresses, such as low and high temperatures or oxidative stress (Timperio *et al.*, 2008). The main functions of Hsp 70 are preventing aggregation of proteins (Sheffield *et al.*, 1990) and assisting refolding of proteins under both normal and stress conditions (Wang *et al.*, 2004); they are also involved in transport and proteolytic degradation of proteins (Fu *et al.*, 2010).

Bromoperoxidase belongs to a particular class of peroxidases, the haloperoxidases, which catalyse the oxidation of halides in the presence of hydrogen peroxide (Leblanc *et al.*, 2006; Cosse *et al.*, 2009). Members of the bromoperoxidases are likely to be involved in anti-oxidant protection in kelps (Roeder *et al.*, 2005; Küpper *et al.*, 2008). Stress conditions, particularly high light and high temperature, lead to an increased expression of stress genes e.g. Hsps and genes with anti-oxidative functions (Collén *et al.*, 2007). The over-representation of these generally stress-associated domains indicates that the corresponding gene families are amplified in the *S. latissima* genome versus that of *E. siliculosus*. The cDNA library also reflects the chosen stress conditions and is therefore an excellent basis for further microarray investigations under relevant conditions.

In conclusion, we present the first large-scale EST study of a kelp, namely *S. latissima*. Of the 28 803 high-quality contigs, we were able to identify over 10 000 contigs either by BLASTx, Interpro protein motif annotation, or Gene Ontology, leading to functional genome coverage of perhaps 70%. Our study has confirmed the EST approach as an efficient and cost-effective tool for gene discovery in non-model organisms. The establishment of this large EST collection is a first step towards designing cDNA microarrays for investigating of gene expression profiles under light and temperature stress in *S. latissima*. Furthermore the data provide an important resource for studies of comparative RNA-seq mapping and annotation of the forthcoming genomic DNA-sequences of *S. latissima* and related kelp species.

Supplementary material

Supplementary material is available for this article via the Supplementary Content tab of the article's online page at

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Transcriptomic analysis of acclimation to temperature and light stress in *Saccharina latissima* (Phaeophyceae)

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Title (150 characters or fewer)
Transcriptomic Analysis of Acclimation to Temperature and Light Stress in *Saccharina latissima* (Phaeophyceae)

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Abstract

Kelps, brown algae of the order *Laminariales*, dominate rocky shores and form huge kelp beds, which provide habitat and nurseries for various marine organisms. Whereas the basic physiological and ecophysiological characteristics of kelps are well studied, the molecular processes underlying acclimation to different light and temperature conditions are still poorly understood. Therefore we investigated the molecular mechanisms underlying the physiological acclimation to light and temperature stress. Sporophytes of *S. latissima* were exposed to combinations of light intensities and temperatures and microarray hybridizations were performed to determine changes in gene expression patterns. This first large-scale transcriptomic study of a kelp species shows that *S. latissim*a responds to temperature and light stress with a multitude of transcriptional changes: up to 32% of genes showed an altered expression after the exposure experiments. High temperature had stronger effects on the gene expression in *S. latissima* than low temperature, reflected by the higher number of temperature responsive genes. We gained insights into underlying molecular processes of acclimation, which includes adjustment of the primary metabolism as well as induction of several ROS scavengers and a sophisticated regulation of Hsps. We show that *S. latissima,* as a cold adapted species, must make stronger efforts for acclimating to high than to low temperatures. The strongest response was caused by the combination of high temperatures with high light intensities, which proved harmful for the alga.

Introduction

Marine macroalgae are key components of coastal ecosystems and play an important role as the nutritional basis in marine communities [1-3]. Although they cover only a

small percentage of the area of the world`s ocean, they account for up to 10% of the global oceanic primary production [4,5]. The largest biogenic structures found in benthic marine systems are kelp beds, which are mostly consisting of macroalgae from the order Laminariales [3]. Kelp beds dominate rocky coastal shores of the world's cold-water marine habitats; they provide a unique three-dimensional habitat for marine organisms by offering a physical structure for shelter, protection from predators and nurseries ground as well as food for various marine animals [2,6]. Kelps are cold-temperate water species; their high abundance and biomass in sub-polar and cold-temperate regions suggests that they are well adapted to low light and low temperatures [1,7,8]. Cold adapted species developed mechanisms, such as changes in gene expression to maintain sufficient rates of enzyme-catalyzed reactions and modifications within the thylakoid membrane system, affecting photosynthetic electron transport, to overcome the constraints of exposure to low temperature [9,10].

The primary abiotic factors responsible for the vertical zonation and geographical distribution of kelps are light, including UV radiation, and temperature [11-13]. Current ocean temperature rises caused by global warming and stratospheric ozone depletion thus likely will influence zonation, distribution patterns, and the performance of kelp at their present habitats. The perennial kelp *Saccharina latissima* (= formerly *Laminaria saccharina*) is a common species in polar to temperate coastal waters and is distributed circumpolar in the northern hemisphere [14,15]. The natural growth sites of *S. latissima* are clear as well as turbid coastal waters, it occurs from the intertidal down to 30m depth; as a result *S. latissima* is exposed to a wide range of temperature and light conditions [16]. Both the wide latitudinal and vertical distribution of this species might be correlated to ecotypic differentiation of populations with respect to light and temperature. Ecotypic differentiation was reported for sporophytes and gametophytes of *S. latissima* from Long Island Sound (USA) and the Atlantic coast from Maine (USA) [16-18] as well as for gametophytes from Spitsbergen (Norway) and Helgoland (Germany) [19]; the variation in light- and temperature related traits is suggested to have a genetic basis.

The effects of the abiotic factors light and temperature on the physiology and growth of *S. latissima* have been studied extensively [7,20-23], yet the involved molecular processes of acclimation and adaption are still poorly understood. Gene expression data for kelps are scarce, until now only three cDNA approaches have been published. Crépineau et al. 2000 characterized 905 expressed sequence tags (ESTs) of life cycle stages of *Laminaria digitata* [24], Roeder et al. 2005 established a cDNA library of 1985 ESTs from *Laminaria digitata* protoplasts [25]. More recently a subtractive cDNA approach on oligoguluonates induced transcriptional defence responses was conducted [26]. For sessile organisms such as kelps acclimation to environmental changes in order to maintain cellular function is particularly important. Extrinsic stress resulting from changes in abiotic factors, e.g. light and temperature, is regarded as the most important stress agent in plants [27].

Excessive light causes photo-oxidative stress through an over-reduction of the photosynthetic electron transport chain; electrons from the light reaction are transferred to oxygen, which leads to the formation of reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxides [28-30]. Low temperature alters significantly plant metabolism, its physiology and plant productivity, given that in general enzyme activity decreases with declining temperatures [31,32]. The reduced activity of the Calvin cycle results in a decrease of production for the final electron acceptor $NADP^+$, which may lead to electron transfer from reduced ferredoxin to oxygen and therefore to the formation of ROS [33]. Heat stress on the one hand leads to the

degradation and dysfunction of proteins, on the other hand to an uncoupling of pathways, resulting in formation of ROS, which – in turn - induce lipid peroxidation [34-37]. Although the abiotic factors light and temperature operate through different mechanisms, both lead to the formation of ROS. ROS cause cellular damage in terms of denaturation of nucleic acids, proteins, polysaccharides and lipids [38-40]. Cells are able to counteract ROS with a sophisticated network of non-enzymatic and enzymatic systems, which scavenge the various ROS intermediates [41-43]. The non-enzymatic antioxidants include the major cellular redox buffers ascorbate (Asa) and glutathione (GSH) [44,45]. Enzymatic ROS detoxification includes the conversion of superoxide radicals into hydrogen peroxide and oxygen by superoxide dismutase (SOD), subsequently hydrogen peroxide is eliminated in the ascorbate–glutathione cycle by ascorbate peroxidase (APX) and glutathione reductase (GR) [43,46,47]. However, ROS are not only potentially harmful, but also part of a subtle network of signaling reactions [48]. ROS as well as the redox state of several regulatory redox-reactive key molecules, such as thioredoxin and glutathione, are signals which regulate expression e.g. of photosynthesis-related genes [33,41].

The following study aimed to investigate the molecular mechanisms underlying the physiological acclimation to temperature and light stress in *S. latissima* from the Arctic (Spitsbergen). In order to analyze the sensitivity of this cold adapted species to either high or low temperatures in combination with high light, sporophytes were exposed for 24h to 5 different combinations of light intensities and temperatures. Photosynthetic efficiency, measured as variable fluorescence of PS II, was determined before and at the end of the experiment. Changes in gene expression levels were assessed through oligonucleotide microarrays. We expected that *S. latissima*, as a cold adapted species, would be more negatively effected by high than low temperatures and that this will be reflected in the photosynthetic fitness and the changing gene expression profiles of the organism.

Results

Photosynthetic efficiency

Initial mean maximum quantum yield of PS II (Fv/Fm) was 0.633 ± 0.02. Maximum quantum yield of PS II remained stable at low photosynthetically active radiation (PAR) under the three tested temperature regimes (control low PAR 12°C; Fv/Fm= 0.653 ± 0.01). A significant decrease ($p < 0.01$) in efficiency of PS II was observed in sporophytes exposed to high photosynthetically active radiation at 2°C (Fv/Fm= 0.308 ± 0.07) and 17°C (Fv/Fm= 0.09 ± 0.003). Strongest photoinhibition occurred under high photosynthetically active radiation and a temperature of 17°C (Figure 1).

Gene expression patterns in response to light and temperature stress

Oligonucleotide microarrays covering 26,224 transcripts were used for determining changes in gene expression patterns under temperature (2°C/17°C) and high photosynthetically active radiation (PAR) stress. Of these 10,915 transcripts (42%) showed different expression patterns under at least one stress treatment compared to the control treatment (12°C & low PAR). The strongest effect on gene expression was observed in the high PAR/17°C treatment when 8,334 genes (32%) were affected. The remaining three treatments (2°C & high/low PAR, 17°C low PAR) caused transcriptional changes for 13-19% (3,289 – 4,920) of the genes (Figure 2).

Identification of temperature and light regulated genes

A cross comparison was performed to identify an overlap of ESTs responsive to either high or low temperature within the different light treatments (Figure 3).
High temperature was found to up-regulate the expression levels of 2,028 genes and down-regulate those of 988. The effect of low temperatures was less pronounced, in that 1,273 genes were found to be up-regulated and 1,002 genes down-regulated. The cross comparison revealed that the amount of high PAR responsive ESTs is dependent on temperature: at high PAR and low temperature 1,456 genes were induced whereas 1,188 genes were repressed; at high PAR and high temperature 2,949 genes were induced and 2,369 genes were repressed.

Gene enrichments were performed to assign biological processes to these large groups of responsive ESTs. We analyzed whether the abundance of certain Gene Ontology (GO) terms within the GO root category biological process is significantly different between the groups of the cross comparison versus the whole gene set on the microarray. Special emphasis was given to the proportion between biosynthetic and catabolic processes within the different stresses. In the group of genes simultaneously up-regulated under both light conditions at low temperature the GO term one-carbon metabolic process (GO:0006730) was over-represented. The high PAR low temperature up-regulated genes revealed 52 enriched GO terms of the roots biological process (for a detailed list see Table S1). Among these more catabolism related GO terms were detected, (e.g. polysaccharide catabolism, glycerolipid catabolism, and triglyceride catabolism) than GO terms related to biosynthesis (e.g. cellular amino acid biosynthesis, cellular nitrogen compound biosynthesis, and nucleobase biosynthetic process). Among the down-regulated genes at low temperature as well as at high PAR and low temperature no over-represented GO terms could be found. The set of down-regulated high temperature responsive ESTs featured 29 over-represented GO categories, representing often catabolic as well as biosynthetic processes of a metabolic process, e.g. monosaccharide biosynthetic process (GO:0046364), monosaccharide catabolic process

(GO:0046365), hexose biosynthetic process (GO:0019319), and hexose catabolic process (GO:0019320). The highest abundance of enriched GO terms was observed among the high PAR high temperature down-regulated genes. We detected more catabolism-related GO terms then biosynthesis-related GO terms. 125 over-represented GO terms were identified, including several crucial metabolism categories e.g. nucleobase, nucleoside and nucleotide metabolic process (GO:0055086), photosynthesis (GO:0015979), alcohol metabolic process (GO:0006066), carbohydrate metabolic process (GO:0005975), and cellular amino acid metabolic process (GO:0006520).

Gene enrichments of single treatment conditions

Gene enrichments were performed for all significantly regulated genes of the different stress conditions. The analyses showed that temperature stress combined with high PAR stress led to a stronger shift in gene expression compared to the control treatment than temperature stress alone. We detected 50 over-represented GO terms among the regulated genes under low temperature and high PAR and 89 enriched GO terms within the high temperature and high PAR regulated genes, but only 5 respectively 4 GO terms within the regulated genes under low and high temperature stress alone. For a detailed list see Table S2.

The high PAR treatments showed 23 common enriched GO terms, e.g. plastid (GO:0009536), oxidation reduction (GO:0055114), regulation of cellular process (GO:0050794), and cellular component organization (GO:0016043). The high PAR treatment at low temperature additionally showed a higher abundance of GO terms correlated to amino acid metabolism such as cellular amino acid biosynthetic process (GO:0008652), cellular amino acid and derivative metabolic process (GO:0006519), and glutamine family amino acid metabolic process (GO:0009064). High PAR treatment at high temperature on the contrary led to an enhanced appearance of GO terms associated with photosynthetic components (e.g. light-harvesting complex GO:0030076, photosystem II GO:00095239), catabolism (e.g. glucose catabolic process GO:0006007, monosaccharide catabolic process GO:0046365), and biosynthetic processes, in particular cellular nitrogen compound biosynthetic process (GO:0044271), porphyrin biosynthetic process (GO:0006779), and macromolecule biosynthetic process (GO:0009059). We found no shared enriched GO terms within the low PAR treatments. At low temperature the terms organelle part (GO:0044422), intracellular organelle part (GO:0044446), protein complex (GO:0043234), and one-carbon metabolic process (GO:0006730) were identified as being over-represented, whereas at high temperatures the terms cellular macromolecule metabolic process (GO:0044260), macromolecule metabolic process (GO:0043170), nucleobase, nucleoside, nucleotide and nucleic acid metabolic process (GO:0006139), nucleus (GO:0005634), and plastid (GO:0009536) occurred more often.

Classification of over-represented KEGG pathways

KOBAS analyses resulted in the identification of several significantly enriched pathways within the different treatments (Table 1). At low temperature in both light conditions up-regulated genes involved in glycine, serine and threonine metabolism were affected. Among the up-regulated genes under low temperature and high PAR we observed additionally an increased regulation of alanine, aspartate and glutamate metabolism and aminoacyl-tRNA biosynthesis. Common features at high temperature included down-regulation of transcripts involved in carbon fixation. Furthermore the high PAR and high temperature condition led to a significant down-regulation of several metabolic

pathways such as collecting duct acid secretion, glycolysis/ gluconeogenesis as well as glutamate, fructose and mannose, nitrogen, and porphyrin and chlorophyll metabolism.

Manual classification of genes responsive to temperature and oxidative stress

To investigate the mechanisms involved in the response to temperature and high PAR mediated oxidative stress, as well as the effects from these stressors on the state of photosynthetic components, we examined manually transcriptional changes of genes encoding for photosynthetic components (Table 2), ROS scavengers (Table 3), heat shock proteins (Hsps) (Table 4), and transcripts correlated to proteolysis (Table 5). A full list of the regulated genes with annotations can be retrieved from the supplemental material (Table S3). We observed a strong down-regulation of transcription for most of the photosynthetic components in response to the high PAR 17°C treatment, the strongest transcriptional changes of up to 61.7-fold occurred in genes correlated to the light harvesting complex (contig15369, contig07943, contig08792). Additionally transcripts encoding for the photosystem II (e.g. photosystem II 12 kDa extrinsic protein, photosystem II cp47 chlorophyll apoprotein, and photosystem II D2 protein), and transcripts of thylakoid luminal proteins (contig07691, contig24607) were significantly repressed. The 17°C low PAR treatment featured significantly down-regulated transcripts encoding for fucoxanthin chl a c lhca clade, light harvesting complex protein 4, light-harvest protein, and photosystem I reaction center subunit XI, for them transcriptional changes between 2.1 – 2.7-fold were observed. The low temperature treatments on the contrary led to a significant induction of photosynthetic component transcripts: some transcripts showed a similar abundance within the high and low light treatment, e.g. light harvesting complex protein and photosystem II protein y. Other up-regulated transcripts at low temperature such as photosystem II biogenesis protein psp29 and thylakoid lumenal protein exhibit higher transcriptional changes within the high PAR treatment compared to the low PAR treatment.

We found a significant transcriptional regulation for several genes encoding antioxidative enzymes. Transcripts encoding for alternative oxidase chloroplastic, glutathione reductase, and thioredoxin reductase showed a significant up-regulation at all stress treatments except at low light/2°C. The highest transcriptional response for the chloroplastic alternative oxidase appeared within the high PAR 17°C treatment, whereas the induction of glutathione reductase and thioredoxin reductase was strongest at the low temperature and high PAR condition. Transcripts of glutaredoxin, glutathione S-transferase, and mitochondrial superoxide dismutase precursor were up-regulated at high temperatures and featured the highest transcript abundances at the high temperature/high PAR condition. Superoxide dismutase [Fe] in contrast was significantly up-regulated at low PAR 17°C, whereas transcripts encoding for l-ascorbate peroxidase featured an induction at high PAR 2°C. Additionally we observed enhanced transcript abundance for chloroplastic thioredoxin at both high PAR conditions. The only significant down-regulation among transcripts correlated to ROS scavenging mechanism was observed for the glutathione S-transferase within the low temperature treatments.

We were able to identify several differently expressed Hsps genes; here we observed a diverse response of transcripts not only to high temperature, but also to low temperature. The strongest induction of Hsps occurred after the exposure to high PAR at 17°C with an 34.2-fold up-regulation for the heat shock 70 kDa protein 5, in addition we observed four chaperones, e.g. the heat shock 70 kDa protein 5 and the mitochondrial heat shock protein mtHSP70, exclusively up-regulated in response to this treatment. The transcript abundances of the chaperone protein DnaJ and the heat shock cognate 70 kDa

protein 3 were significantly enhanced after all treatments except at the high temperature low PAR treatment, whereas chaperone protein Dnak, heat shock 70 kDa protein 5, and heat shock-like 85 kDa protein were up-regulated at all conditions except the low temperature low PAR condition, the heat shock 70 kDa protein 5 and the heat shock-like 85 kDa protein featured a stronger up-regulation at 17°C. Three Hsps, heat shock protein 33 homolog, heat shock protein 60, and heat shock protein 90 were significantly up-regulated in response to low temperature stress at both light conditions, but with a stronger induction at high PAR 2°C. Transcripts of the mthsp70-associated motor and chaperone protein exhibit a significantly higher abundance within the high temperature treatments with a stronger expression after the high PAR 2°C treatment.

We detected a significant induction of the 26s protease regulatory subunit 6a, 6b, and 8a at high PAR 17°C, whereas the proteasome subunit alpha type 2 was induced in both high temperature treatments. All genes belonging to the process of ubiquitin mediated proteolysis, except for the ubiquitin-like modifier-activating enzyme 1, were exclusively up-regulated at high PAR 17°C. The ubiquitin-like modifier-activating enzyme 1 was up regulated in both high temperature treatments, but featuring an enhanced up-regulation within the high light condition. Transcripts annotated as an ATP-dependent protease ATPase subunit and a cathepsin b-like cysteine proteinase 5 were induced at high PAR 17°C, whereas transcripts for the ATP-dependent clp protease proteolytic subunit was found to be up-regulated at high PAR 2°C. Interestingly we also detected two transcripts belonging to the family of cysteine proteases; the transcript abundance of a cysteine proteinase 2 was significantly enhanced at high PAR 17°C, while the transcript of a papain family cysteine protease containing protein was 2.3- fold induced at low light 17°C and 9.2-fold induced at high light 17°C.

Discussion

This study represents the first broad scale gene expression study of a kelp species in response to abiotic stress covering about 70% of the genome. Our aim was to investigate expression profile changes in *Saccharina latissima* after exposure to high PAR and temperature stress. We observed a multitude of transcriptional changes: Up to 30% of genes showed an altered expression after the exposure experiments. Similarly, in *Chondrus crispus* 25% of the studied genes exhibited transcriptional changes after exposure to different abiotic stressors [49]. In *Ectocarpus siliculosus,* almost 70% of the expressed genes featured significant changes in transcript abundance in response to stress [50] We found that more genes were differently up-regulated than down-regulated, additionally more genes were regulated at high than at low PAR conditions; the largest amount of transcriptional changes was observed at high PAR and 17°C.

Photosynthesis

Sporophytes exposed to high photosynthetically active radiation conditions showed a significant decrease in the maximum quantum yield of PS II. The combination of high PAR and a temperature of 17°C resulted in the highest degree of photoinhibition, showing 90% reduced Fv/Fm as compared to the control after 24h of exposure. This was reflected by strong down-regulation (up to 60-fold) of transcripts responsible for photosynthetic components, e.g. encoding for light harvesting complex proteins, photosystem II related proteins, porphyrin and chlorophyll metabolism proteins, and carbon fixation enzymes. Fluorescence measurements as well as gene expression studies indicate a breakdown of photosynthesis. Thus we suggest that *S. latissima* from the Arctic is particular susceptible to chronic photoinhibition at high PAR high temperature conditions. High PAR at 2°C caused a decrease in maximum quantum yield between 40-50% compared to the control, on the transcriptional level we observed an up-regulation between 2-5 fold of photosystem II, thylakoid, and light harvesting complex protein correlated transcripts. The low PAR treatments on the contrary induced no significant changes in maximum quantum yield of photosystem II, nevertheless light harvesting complex transcripts as well as some of the photosystem II transcripts were up-regulated at 2°C and down-regulated at 17°C with a fold change of about 2. Our results are in agreement with physiological studies showing that *Saccharina latissima* is well adapted to low temperature and low light conditions [13,17,51-53]. The observed effect of high PAR stress studies on the photosynthesis of *S. latissima* might be due to the shade adaption of the species, which is expressed in a low compensation point, high absorbance and photosynthetic efficiency (α) especially in shade grown plants [54,55], a physiological constitution favoring strong photoinhibition when exposed to high PAR [56]. Low light adapted subtidal algae are more sensitive to high PAR stress than high irradiance adapted algae [57]. Another study revealed that high temperatures can enhance photodamaging effects of high PAR in plants [58]. In *Saccharina latissim*a photosynthetic efficiency decreases with increasing temperatures [7]. However, hyper-optimal temperatures of up to 22°C did not influence photoinhibition in *S. latissima* [59]. These contradicting results might be due to ecotypic differentiation within the species, in turn explaining the huge capacity for climatic adaption and therefore its wide geographic distribution.

The observed regulation of transcripts encoding for light harvesting complex (LHC) in all our experimental conditions leads to the suggestion, that the regulation of LHC proteins is a prominent part of the photoacclimatory mechanism in *S. latissima*. This agrees with former studies, showing that changes in light availability and

temperature, as well as the age of the thalli, influenced the pigment content and composition in *S. latissima* [7,20,21]. The expression of the nuclear encoded LHC genes is regulated by chloroplast redox signals, including the redox state of electron transport components [33] as well as chlorophyll biosynthetic intermediates [60]. The abundance of transcripts correlated to photosystem II varied within the stress treatments, with a trend towards up-regulation at low temperatures and down-regulation at high temperatures. The photosystem II biogenesis protein psp29 was up-regulated at both low temperature conditions with higher transcript abundance at high PAR. Disruption of this gene results in an impairment of PS II function under high irradiance confirming the involvement of psp29 in PS II biogenesis and disintegration following photodamage [61]. Hence the up-regulation of this gene at low temperatures might be due to a cold shock photoinhibition effect, as temperature leads to an increase of photo-oxidative stress, partly because of the reduced activity of the Calvin cycle [33,62].

Furthermore we detected specific transcriptional changes for those treatments causing decreased maximum quantum yield: photosystem II D2 protein was up-regulated at high PAR 2°C, whereas photosystem II reaction center protein D1/psbA was strongly down-regulated at high PAR 17°C. Photoinhibition is a state of physiological stress that appears when excessive light is absorbed by the photosynthetic apparatus; chronic photoinhibition occurs when the rate of damage exceeds the capacity of the PSII repair and is associated with the inactivation and degradation of PS II reactions centres [20,63-65]. The recovery mechanisms of photosystem II include the removal of damaged D1 proteins and replacement by newly synthesized molecules for the PSII [66]. Recent studies by [67] revealed that photodamage of PS II is not only associated with damage within the PSII reaction center, but also with a depressed repair rate caused by inhibition of the synthesis of the D1 protein at the step of protein translation. Our finding of strongly lowered maximum quantum yield and reduced transcript abundance of D1 therefore suggest that the severe damage to PS caused by the 17°/high PAR treatment could not be overcome by transcriptional regulation in *S. latissima*.

General stress response characteristics

S. latissima exhibited significant changes in gene expression after the exposure to different temperatures, even though no influence on the maximum quantum yield of photosynthesis was observed. The higher number of high temperature responsive genes indicates that high temperature had stronger effects on the gene expression in *S. latissima* than low temperature. This implies that high temperatures are more harmful for *S. latissima* than low temperatures, resulting in stronger efforts to overcome the negative effects. Gene enrichment analysis showed down-regulation of carbohydrate biosynthetic and catabolic processes after exposure to high temperature, as well as repression of transcripts correlated to photosynthesis and carbon fixation. A possible explanation might be the increasing reaction rate of enzymes at elevated temperatures. In many cellular processes the reaction rate tends to increase with a Q_{10} from about 2 [68]. Furthermore, a down-regulation of transcripts can partially be caused by energetic and mass limitations of the full transcriptome, favouring transcripts of acute and chronic stress response.

At 2°C we detected up-regulation of genes correlated with the metabolism of glycine, serine and threonine. An increase in the amino acid content during cold acclimation was also demonstrated for the green algae *Klebsormidium flaccidum* [69]. Only few studies were conducted on the amino acid metabolism of macroalgae [70,71] and until now no data are available on the influence of cold acclimation on the amino acid metabolism of macroalgae. Serine is involved in the final step of cysteine

biosynthesis, which includes incorporating of a sulphide moiety and an amino acid moiety from serine through O-acetyl-L serine [72]. The amino acids cysteine, glycine and glutamate are essential for synthesis of Glutathione (GSH), a reducing co-factor for several enzymes involved in ROS detoxification [48,73]. Furthermore it was observed that GSH levels respond to the availability of cysteine and glycine, an elevated capacity for GSH synthesis in the chloroplast can influence metabolic upstream events for satisfying the increased substrate demand [74]. The up-regulation of glycine, serine and threonine metabolism in *S. latissima* at low temperature thus might be reflecting the higher demand of GSH due to an increase of photooxidative stress.

The interactive effects of temperature and light stress on photosynthesis in *S. latissima* were also observed at the transcriptional level; the amount of high PAR responsive ESTs doubled at high temperature. In general high PAR caused an up-regulation of catabolic processes for energy supply as well as for genes with antioxidant functions. The combination of light and low temperature stress led on the one hand to an induction of lipid catabolism and carbohydrate metabolism, on the other hand to an increased number of induced gene activities in cellular amino acid biosynthesis, cellular nitrogen compound biosynthesis and nucleobase biosynthesis. Furthermore we observed enhanced up-regulation of the aminoacyl-tRNA metabolism. These results suggest that energy was generated for removal and replacement of damaged proteins as well as for the synthesis of stress related genes, e.g. Hsps and ROS scavenging enzymes. Cells are able to balance finely the expression of stress related and growth related genes. Stress results in an induction of genes with energy generating, heat shock and antioxidant functions, whereas growth related genes are mostly repressed [75]. Few studies focused on molecular stress response patterns in other macroalgae. [76] Collén et al. 2006 examined the gene expression profiles of *Chondrus crispus* after exposure to methyl jasmonate that resulted in a repression of genes involved in energy conversion and general metabolism and to an induction of stress-related genes, e.g. glutathione S-transferase and Hsp-20. In another study Collén et al. 2007 investigated the response of the transcriptome of *Chondrus crispus* to high light, high temperature and osmotic stress. At this a decreased expression of energy- and protein synthesis and increased expression of stress genes was observed, suggesting that available resources were rather used for reducing potential damage and repair of structure than for growth related processes [49]. A recent study of global gene expression in *Ectocarpus siliculosus* in response to abiotic stress gave similar results, namely repression of genes related to growth and primary metabolism and induction of energy generation and production of protectants [50].

Excessive light at high temperature was the most destructive stress condition for *S. latissima*, resulting in a strong down-regulation of several metabolic processes, e.g. photosynthesis, carbohydrate metabolism and amino acid metabolism. We detected enhanced expression of ROS scavengers as well as a high abundance of Hsp transcripts. A striking feature of the high PAR/high temperature treatment was the significant induction of several genes involved in proteolysis, e.g. protease regulatory subunits and components of the ubiquitin-mediated proteolysis, which did not occur in any other of our stress treatments. Our data suggest that this treatment caused the highest degree of protein dysfunction. The 26S proteasome is an essential part of the ATP-dependent proteolysis in eukaryotes, consisting of two functionally different sub-complexes, the 20S core protease and a 19S regulatory particle [77,78]. It is responsible for the degradation of various cellular proteins, including also critical regulatory proteins (e.g. cyclins) as well as transcription factors; hence it is involved in various cellular processes, for instance apoptosis, metabolic regulation and signal transduction [79,80].

Interestingly the induction of two genes annotated as different cysteine proteases, cysteine proteinase 2 and papain family cysteine protease containing protein, was observed in *S. latissima* under excessive light and high temperature. Cysteine proteases are involved in the intracellular protein degradation, they respond to different internal and external stimuli and are able to provide to 90% of the proteolytic activity [81,82]. Moreover, several studies showed that the cysteine protease activity is a key event in programmed cell death in plants and different phytoplankton species [83-86]. Taken all together, our data indicates that *S. latissima* is not able to adapt to excessive light in connection with high temperatures, but rather responses with programmed cell death to this stress.

ROS scavenging enzymes

We detected significant induction of several transcripts correlated with reactive oxygen species (ROS) scavenging mechanisms. Transcripts encoding for the superoxide dismutase (SOD) [Fe] were induced at low PAR 17°C, whereas transcripts encoding for a mitochondrial SOD precursor were up-regulated in both light conditions at 17°C. ROS species such as superoxide and hydrogen peroxide are involved in response to biotic and abiotic stress in macroalgae [38,87-89]. SOD catalyzes the reaction from superoxide anion to hydrogen peroxide and oxygen [28,90,91]. A couple of studies focused on the SOD enzyme activity in response to abiotic stresses in macroalgae, Bischof et al. (2003) detected an increased enzyme activity of SOD in *Ulva* after exposure to PAR and UV irradiance [28], the same was shown for *Chaetomorpha linum* after exposure to UV radiation [92]. An increased SOD activity was also observed in *Ulva fasciata* in response to salinity stress [93]. Gene expression analysis of *Chondrus crispus* indicated an up-regulation of SOD transcripts after exposure to methyl jasmonate [76]. Since the generation of ROS increases at low temperature [42] the observed up-regulation of SOD at high temperature in our experiments seems to be counterintuitive at first sight. However, our results agree with other investigations: Vyas & Kumar (2005) analyzed the activity of SOD from tea (*Camellia sinensis*) over a wide temperature range and detected increased activity of SOD at decreasing temperatures with an optimum at 0°C. This peculiarity was also shown for several phytoplankton species by Perelman et al. (2006), which suggested that the benefit of this low temperature activation provides a faster protection than de novo enzyme synthesis only [31].

We observed significant induction of genes coding for enzymes of the ascorbate-glutathione cycle. Ascorbate peroxidase (APX) was induced after exposure to high PAR at 2°C, whereas the glutathione reductase (GR) was up-regulated in all treatments except low PAR at 17°C, with the strongest induction after exposure to high PAR at 2°C. Additionally, a higher number of transcripts encoding for glutaredoxin was found in response to high temperature. The ascorbate-glutathione cycle is considered to play an important role in ROS scavenging [45]. In this pathway reduced glutathione (GSH) is required for the reduction of dehydroascorbate, which is generated via monodehydroascorbate by APX activity [94]. Glutaredoxins are small heat-stable disulphide oxidoreductases, which catalyze glutathione–dependent reactions and are suggested to protect cells against oxidative damage [95,96]. However, plants contain various glutaredoxins whose functions are still unknown [97]. Some glutaredoxins were shown to be up-regulated by heat stress [98], furthermore there is evidence that glutaredoxins interact with Hsps and antioxidant enzymes [99,100].

In macroalgae the activity of ROS scavenging enzymes has been studied extensively. Macroalgae exhibit increased activity of GR and APX after exposure to various abiotic factors such as low temperature [101], UV radiation [47,89], copper

concentration [38,46] and desiccation [94]. Only a few projects focused on gene expression in macroalgae during stress. Collén *et al.* 2007 observed the over expression of APX transcripts in *Chrondrus crispus* after exposure to high light conditions [49]. The green alga *Ulva fasciata* showed enhanced up-regulation of APX and GR genes in response to copper stress [46]. Dittami *et al.* (2009) detected enhanced expression of glutaredoxin and glutathione peroxidase in *Ectocarpus siliculosus* after exposure to oxidative and hyper saline stress, respectively [50]. Our results conform to former studies, proving the ascorbate-glutathione cycle being an important anti-oxidant mechanism in macroalgae.

Two genes of the chloroplastic thioredoxin system were detected among the significantly up-regulated ones. Transcripts of chloroplastic thioredoxin showed enhanced abundance at high PAR conditions, whereas thioredoxin reductase was induced after all treatments, except for low PAR at 17°C, with highest fold change detected after exposure to high PAR at 2°C. Thioredoxins (Trx) are low molecular weight thiol-disulphide oxidoreductases and involved in redox homeostasis [102,103]. Thioredoxin reductase is an abundant thiol based peroxidase, which catalyzes the reduction of thioredoxin [104]. Two forms of thioredoxin reductase are present in the chloroplast, which use either ferredoxin or NADPH as an electron donor [102]. Plastidic thioredoxins are induced in response to high light intensities, indicating a function in redox balancing related to photosynthesis [105], are regulating via ferredoxin-thioredxin reductase photosynthetic enzymes by light, and are critical for redox regulation of protein function and signaling via thio redox control [106]. Furthermore studies by [107] suggest a complex interaction between Trx, glutaredoxins and glutathione, which are key components of the cellular redox-signaling network. Taken together it can be concluded that *S. latissima* possesses strong protection mechanisms against oxidative damage. ROS scavenging mechanisms showed the strongest induction after exposure to high light at 2°C, suggesting that the highest amount of ROS is generated under excessive light in combination with low temperature.

Heat shock proteins

We observed differential up-regulation of various heat shock proteins (Hsps). Members of the Hsp 70 family were most strongly expressed after the high PAR 17°C treatment. Interestingly three Hsps, Hsp 33, Hsp 60, and Hsp 90, respectively, were exclusively induced at low temperatures. Another four transcripts (Heat shock 70 kda protein 4, Heat shock cognate 70 kda protein 1, Heat shock factor-binding protein 1, mitochondrial heat shock protein mthsp70) were solely up-regulated in response to high temperature in combination with high PAR. Hsps are highly conserved proteins, which are not only important for a broad array of normal cellular processes, but more so play a crucial role in response to stress by re-establishing functional protein conformation and thus cellular homeostasis [108-110]. We detected members of four families, small heat shock proteins (sHsps), Hsp 60, Hsp 70, and Hsp90, among up-regulated genes. Until now, little is known about the function of Hsps in macroalgae in response to stress. Most studies focused on the expression of Hsp 70, which was shown to be heat shock induced in the red alga *Plocamium cartilagineum* [111], the brown alga *Laminaria japonica* [110] and the green alga *Ulva pertusa* [112]. Collén *et al.* 2007 detected in the red algae *Chrondrus crispus* an enhanced transcript abundance of Hsp 70 in response to high light and high temperature, furthermore a high light dependent induction of the Hsp 90 was observed [49]. One study investigated the survival under heat stress in intertidal embryos of *Fucus spp.*; here the Hsp 60 showed a higher expression level at elevated temperatures [113]. It was recently shown by Pearson et al. (2010) that temperature stress led to an

enhanced expression of several different Hsps in *Fucus*, including the Hsp 18, Hsp 70, Hsp 83 as well as the Hsp STI [114]. Our data suggests that the sophisticated regulation of Hsps in *S. latissima* is a prominent part of acclimation not only to temperature but also to combined environmental stresses like high PAR in combination with high temperature.

Conclusion

The present study shows that *S. latissim*a from the Arctic (Spitsbergen) responds to high temperature and light stress with a multitude of transcriptional changes and that high temperature (17 °C) had stronger effects on the gene expression in *S. latissima* than low temperature (2 °C). We conclude, that *S. latissima,* as a cold adapted species, requires a larger scale metabolic reorganization for acclimating to high temperatures than to low temperatures. For all parameters measured, the combination of high temperatures with high light intensities caused the strongest response and proved extremely harmful for the alga; even leading to an up-regulation of programmed cell death related genes. The combination of the stress factors light and temperature led to interactive effects on photosynthesis and gene expression profiles. Thus simultaneous influence of several stress factors can elevate their damaging effects, and might lead to an increase of susceptibility to additional stresses [115,116]. Possible consequences of this reduced resilience for kelps include local extinctions of population near the southern distribution limit as well as a shift in its distribution towards the Arctic [117]. Future studies should therefore combine more and additional factors, e.g. enhanced CO_2 concentrations, changing salinity, or enhanced UV radiation. With respect to the ecotypic differentiation within this species, similar studies on gene expression under abiotic stresses should be conducted with *S. latissima* populations of the southern distribution boundary, to gain further insights into variability and acclimation potential among spatially separated populations.

Materials and Methods

Culture conditions and stress treatments

Saccharina latissima (Lane) sporophytes were raised from gametophyte cultures, which were established from spores of fertile sporophytes collected by SCUBA diving in Kongsfjorden, (79°N; 11°E; Svalbard, Norway; AWI culture numbers: 3123, 3124). Male and female gametophytes were fragmented together, transferred to petri dishes filled with Provasoli enriched Seawater (PES) [118] and cultured at 10 ± 1°C and 30 µmol photons m^2 s^{-1} photosynthetically active radiation (PAR) at 18h light : 6h dark period . After 2 weeks developing sporophytes were transferred to aerated 5l culture bottles and grown in PES until they reached a size of 5-7cm. The medium was changed twice per week.

Young sporophytes were exposed for 24h in environmentally controlled rooms set to 2, 12, and 17°C ± 1°C to high photosynthetically active radiation (107.8 ± 5 µmol photons m^2 s^{-1}) and low photosynthetically active radiation (23.8 ± 3.1 µmol photons m^2 s^{-1}). All experiments were conducted with five replicates. Photosynthetically active radiation (PAR) was provided by Osram daylight fluorescent tubes (Biolux, 36W; Osram, Germany), and was determined by using a LI- 250 light meter (LI-COR, Lincoln; USA).

Fluorescence measurements

The photosynthetic efficiency was measured for observing the extent of photoinhibition in response to photosynthetically active radiation (PAR) and temperature stress. The maximum quantum yield of PS II (Fv/Fm) was determined in the beginning and at the end of the experiment by use of an Imaging PAM (Pulse Amplitude Fluorometer, Walz, Effeltrich, Germany). Sporophytes were dark-adapted for 5 min prior to the measurements. After the fluorescence measurements the sporophytes were frozen in liquid nitrogen and stored at -80°C until further use.

Fv/Fm values of sporophytes obtained under the different conditions were analyzed using a two-way ANOVA with repeated measurements ($p < 0.01$). Significant differences and interaction of means were compared with the post hoc Tukey test (HSD, $p < 0.01$). All statistical analyses were done using SPSS software version 19 (IBM, USA).

RNA Extraction

Frozen sporophytes were ground in liquid nitrogen and transferred to 2.0 ml Eppendorf tubes. 1 ml extraction buffer (2% CTAB, 1 M NaCl, 100 mM Tris pH 8, 50 mM EDTA, pH 8) and 20 µl DTT 2M were added and mixed well. The mixture was incubated at 45 °C for 10 min. One volume of chloroform: isoamylalcohol (24:1) was added and mixed vigorously for 10 min. The tubes were centrifuged for 20 min at 20°C and 12 000 g. 750 µl of the aqueous phase were transferred into a new tube. 0.3 volumes of EtOH 100% were added and mixed gently by inverting the tube. One volume of chloroform: isoamylalcohol (24:1) was added and a second chloroform extraction followed. 500 µl of the supernatant were transferred to a new cup and total RNA was extracted using a Quiagen Plant Mini Kit (Qiagen, Hildesheim; Germany) according to manufactures protocol for RNA Extraction including on-column DNA-digestion. Quantity and purity of the extracted RNA was determined by a NanoDrop ND-1000 spectrometer (PeqLab, Erlangen, Germany). For verifying the integrity of the RNA the RNA Nano Chip Assay with the 2100 Bioanalyzer device (Agilent Technologies, Böblingen, Germany) was performed.

Microarray design and hybridization

2x105 k microarrays slides were designed with Agilent's eArray online application tool containing 60mer oligonucleotides probes, which were designed from a *Saccharina latissima* cDNA library, thereby 26,224 transcripts were represented by 3 individual probes. The cDNA library, featuring functional genome coverage of approximately 70%, was established from RNA sampled under various light and temperature regimes [119]. Original sequencing files of the cDNA library were uploaded at the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra; accession number SRR305166). Transcripts of the cDNA library were annotated with Blast2GO [120] and blasted against NCBI non-redundant protein database (http://blast.ncbi.nlm.nih.gov/Blast.cgi, NCBI-nr) and the Swiss-Prot protein knowledgebase (http://www.uniprot.org/) (release 2010_7) using the BLASTX algorithm with an e-value cut-off of 10^{-7}.

Total RNA of stress treatments (low PAR 2° and 17°C, high PAR 2° and 17°C) was hybridized against the control treatment (low PAR 12°C); hybridizations were carried out in 4 replicates. For total RNA labeling the Agilent two-color low RNA Input Linear Amplification kit (Agilent Technologies, Waldbronn, Germany) was used. RNA from control treatments was labeled by fluorescent complementary RNA (cRNA) synthesis with cyanine-3-CTP, stress treatment RNA was labeled with cyanine5-CTP.
Prior to the labeling, the Agilent RNA Spike-In Mix (Agilent) was added to 700 ng of total RNA. Due the extensive length of 3`untranslated regions (UTRs) occurring in brown algae cDNA synthesis was performed using a blend of T7 promoter primer and T7 nonamer primer used in equal molarity. cRNA synthesis and purification of labeled RNA was performed following the Agilent Low RNA Input Linear Amplification Kit protocol (Agilent). cRNA yield and specific activity of cyanine-3 and cyanine-5 was determined using the NanoDrop ND-1000 spectrometer (PeqLab, Erlangen, Germany).
Hybridizations were performed with 825ng of cyanine-3 and cyanine-5 labeled cRNA for 17h at 65°C. Afterwards microarray disassembly and wash procedure followed according to manufacturer's instructions (Agilent). Microarrays were scanned with the Agilent G2565AA scanner. Raw data processing was carried out with the Agilent Feature Extraction Software version 9.1.3.1 (FE), for quality monitoring of the microarrays the Agilent QC Tool (v1.0) with the metric set GE2_QCMT_Feb07 was used. The microarray design, raw data and normalized data as well as the detailed experimental design are MIAME compliant and deposited in a MIAME compliant database (ArrayExpress at the EBI; http://www.ebi.ac.uk/microarray-as/ae/; ID: E-MEXP-3450).

Statistical analysis of microarray data

Differential gene expression was analyzed using the GeneSpring GX software platform version 11 (Agilent) with the implemented statistical tests. An ANOVA was performed, followed by a post hoc test Tukey HSD with the Benjamini Hochberg FDR correction. Genes were considered to be differentially expressed when p-Values were less than 0.01 and the calculated fold changes between the control and the treatment exceeded a value of 2.
To perform statistical assessments of GO annotations, whose abundance is significantly different between the regulated genes within the various exposure treatments and the whole microarray, gene set enrichment analysis were done using Blast2GO [120]. Blast2GO employs the Fisher's exact test including corrections for multiple testing using FDR (false discovery rate), FWER (family-wise error rate) and single test *p*-value ($p < 0.01$). Additionally significantly enriched KEGG pathways were identified with KOBAS (http://kobas.cbi.pku.edu.cn/home.do) using a hypergeometric test ($p < 0.01$).

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Figure Legends

Figure 1. Efficiency of PS II (*Fv/Fm*) after 24h exposure to different temperature and radiation conditions. Standard deviations are represented by vertical bars (n=5). Asterisks mark significant differences in efficiency of PS II (two-way ANOVA with repeated measurements, $n = 5$, $p < 0.01$; post hoc Tukey test HSD, $p < 0.01$).

Figure 2. Numbers of differentially up- (black bars) and down-regulated (grey bars) genes after various stress treatments. Identification of regulated ESTs is based on microarray hybridizations and evaluated with an ANOVA against a control treatment with $n = 4$ and $p < 0.01$, followed by a post hoc Tukey test (HSD, $p < 0.01$).

Figure 3. Venn diagram of responsive ESTs after 24h exposure to different light and temperature conditions. Numbers of responsive ESTs are seperated in up (↑) and down (↓) regulated ESTs. The intersections display the number of ESTs regulated in both treatments.

Supplemental information

Table S1. Full list of over-represented Gene Ontology terms for all sections of the Venn diagrams.

Table S2. Full list of over-represented Gene Ontology terms for all stress conditions.

Table S2. Full list of regulated ESTs with annotations for all stress conditions.

Tables

Table 1. Over-represented KEGG pathways among the significantly up- and down-regulated genes in different stress conditions.

Treatment	Regulation	KEGG pathway	KO Id
2°C high PAR / 2°C low PAR	***up***	Glycine, serine and threonine metabolism	ko00260
2°C high PAR	***up***	Alanine, aspartate and glutamate metabolism	ko00250
		Aminoacyl-tRNA biosynthesis	ko00970
17°C high PAR / 17°C low PAR	***down***	Carbon fixation in photosynthetic organisms	ko00710
17°C high PAR	***down***	Collecting duct acid secretion	ko04966
		Glycolysis / Gluconeogenesis	ko00010
		Fructose and mannose metabolism	ko00051
		Nitrogen metabolism	ko00910
		Porphyrin and chlorophyll metabolism	ko00860

Enriched KEGG pathways were identified with a hypergeometric test ($p < 0.01$).

Table 2. Differential regulated genes encoding for photosynthetic components.

Contig name	Putative gene product	Annotation e-Value	Fold change			
			2°C low PAR	2°C high PAR	17°C low PAR	17°C high PAR
Contig12790	Cytochrome b6-f complex iron-sulphur	1.9e-55	-1.1	-1.2	-1.3	**-4.5**
Contig06404	Fucoxanthin-chlorophyll a-c binding protein	1.6e-82	1.1	-1.1	-1.6	**-8.6**
Contig15369	Fucoxanthin chl a c lhca clade	6.9e-59	-1	-1.8	**-2.1**	**-61.7**
Contig12435	Light harvesting complex protein	1.2e-52	**2.1**	**2.9**	1.1	1.2
Contig07943	Light harvesting complex protein 4	5.6e-66	-1.1	-1.5	**-2.6**	**-49.7**
Contig08792	Light-harvest protein	6.4eE-85	-1.5	-1.7	**-2.7**	**-16.5**
Contig00811	Photosystem I reaction center subunit xi	8.9e-40	-1	1.2	**-2.2**	**-8.9**
Contig06470	Photosystem II 12 kDa extrinsic protein	2.4e-49	-1.1	-1.3	-1.5	**-8.9**
Contig02889	Photosystem II biogenesis protein psp29	2.1e-76	**2.8**	**5.4**	-1.1	-1.1
Contig01925	Photosystem II reaction centre protein D1/psba	0	1.7	1.5	1.2	**-3.7**
Contig02559	Photosystem II cp47 chlorophyll apoprotein	0	1.4	1.3	-1.9	**-20.9**
Contig03429	Photosystem II D2 protein	0	1.3	1.9	-1.3	**-14.4**
Contig14092	Photosystem II protein	7.5e-89	1.4	**2.6**	-1.7	**-2.9**
Contig13305	Photosystem II protein y	2.6e-07	**2.6**	**2.4**	1.9	-1.8
Contig28409	Photosystem II stability assembly factor	5.7e-73	1.9	**2.6**	-1.3	-1.8
Contig02889	Thylakoid protein	6.4e-14	**2.8**	**5.4**	-1	-1.1
Contig05910	Thylakoid lumenal 17.4 kDa	1.1e-24	1.5	**2.2**	-1.4	-1.7
Contig07691	Thylakoid lumenal 15 kDa protein	2.5e-36	**2.7**	**4.7**	-1.4	**-2.3**
Contig24607	Thylakoid lumenal protein	1.1e-13	1.1	1.4	-1.7	**-3.1**

All displayed genes were differentially expressed with $p < 0.01$ and were considered to be significant differently expressed with a fold change > 2 (numbers in bold).

Table 3. Differential regulated genes encoding for ROS scavenging enzymes.

Contig name	Putative gene product	Annotation e-Value	Fold change			
			2°C low PAR	2°C high PAR	17°C low PAR	17°C high PAR
Contig09006	Alternative oxidase chloroplastic	9e-46	**2**	**3.1**	1.7	**3.9**
Contig21020	Glutaredoxin	8.6e-09	-1.6	1.8	**2.4**	**2.8**
Contig03562	Glutathione reductase	8.3e-112	**2.8**	**5.1**	1.4	**2.1**
Contig03637	Glutathione S-transferase	1.3e-80	**-5.6**	**-2.8**	**5.2**	**9**
Contig00051	L-ascorbate peroxidase	1.9e-112	1.8	**2.5**	1.5	1.2
Contig05892	Superoxide dismutase [Fe]	1.3e-69	-1.2	-1.3	**2**	1.9
Contig11011	Superoxide dismutase mitochondrial precursor	3.7e-88	-1	1.1	**3.2**	**5.2**
Contig14359	Thioredoxin chloroplastic	8.4e-25	1.9	**2.6**	1.8	**2.9**
Contig08467	Thioredoxin reductase chloroplastic	9.2e-36	**6.1**	**9.4**	1.9	**3.8**

All displayed genes were differentially expressed with $p < 0.01$ and were considered to be significant differently expressed with a fold change > 2 (numbers in bold).

Table 4. Differential regulated genes encoding for heat shock proteins.

Contig name	Putative gene product	Annotation e-Value	Fold change			
			2°C low PAR	2°C high PAR	17°C low PAR	17°C high PAR
Contig12085	Chaperone protein DnaJ	6.5e-56	**2.2**	**2.6**	1.9	**2.2**
Contig01237	Chaperone protein DnaK	5.8e-144	-1.3	**2.1**	**8.8**	**11.2**
Contig06341	Heat shock 70 kda protein 4	4.4e-34	1.2	1.4	1.6	**3**
Contig24742	Heat shock 70 kda protein 5	2.5e-37	1.9	**2.5**	**4.4**	**34.2**
Contig28226	Heat shock cognate 70 kda protein 1	4.9e-66	-1.1	1	1.8	**3.4**
Contig06811	Heat shock cognate 70 kda protein 3	3.6e-59	**2**	**2.1**	1.1	**2.4**
Contig13216	Heat shock factor-binding protein 1	1.5e-11	-1.1	-1.1	1.2	**3.4**
Contig10777	Heat shock protein 33 homolog	1.9e-33	**2.4**	**3.6**	-1.4	**-2.6**
Contig10085	Heat shock protein 60	7.7e-42	**2.5**	**3.7**	1.3	1.4
Contig09075	Heat shock protein 90	2.5e-145	**2**	**4**	1.9	1.8
Contig11643	Heat shock protein mitochondrial mthsp70	1.5e-07	-1.3	-1	1.5	**2.7**
Contig09074	Heat shock-like 85 kda protein	3.3e-09	1.2	**2.4**	**2**	**2**
Contig04604	Mthsp70-associated motor and chaperone protein	1.2e-07	1.1	1.5	**2.4**	**6**

All displayed genes were differentially expressed with $p < 0.01$ and were considered to be significant differently expressed with a fold change > 2 (numbers in bold).

Figures

Figure 1. Efficiency of PS II (*Fv/Fm*) after 24h exposure to different temperature and radiation conditions.

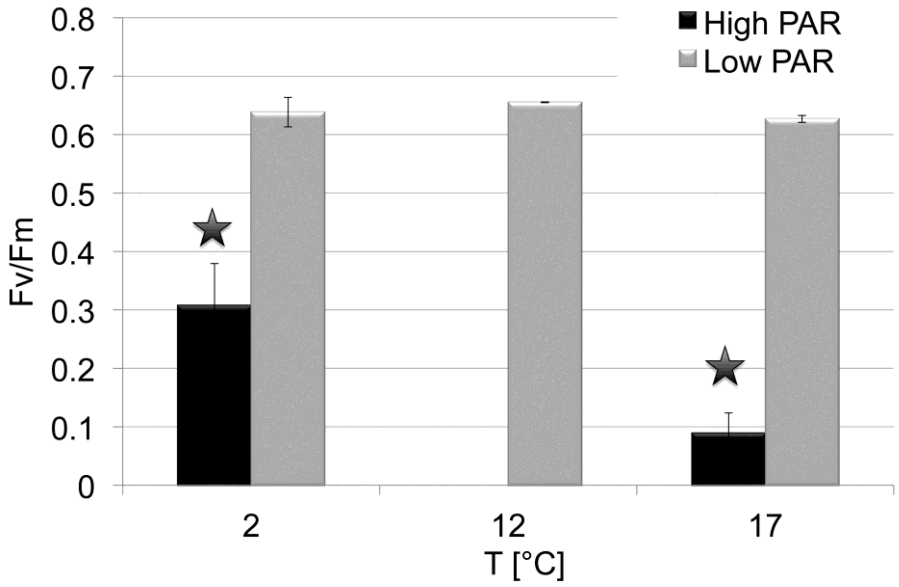


Figure 2. Numbers of differentially up- (black bars) and down-regulated (grey bars) genes after various stress treatments.

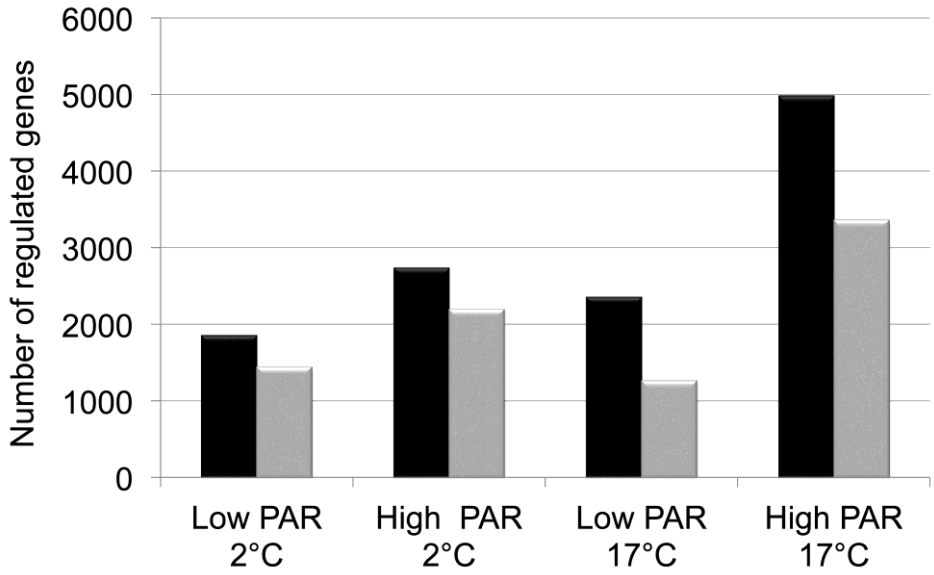
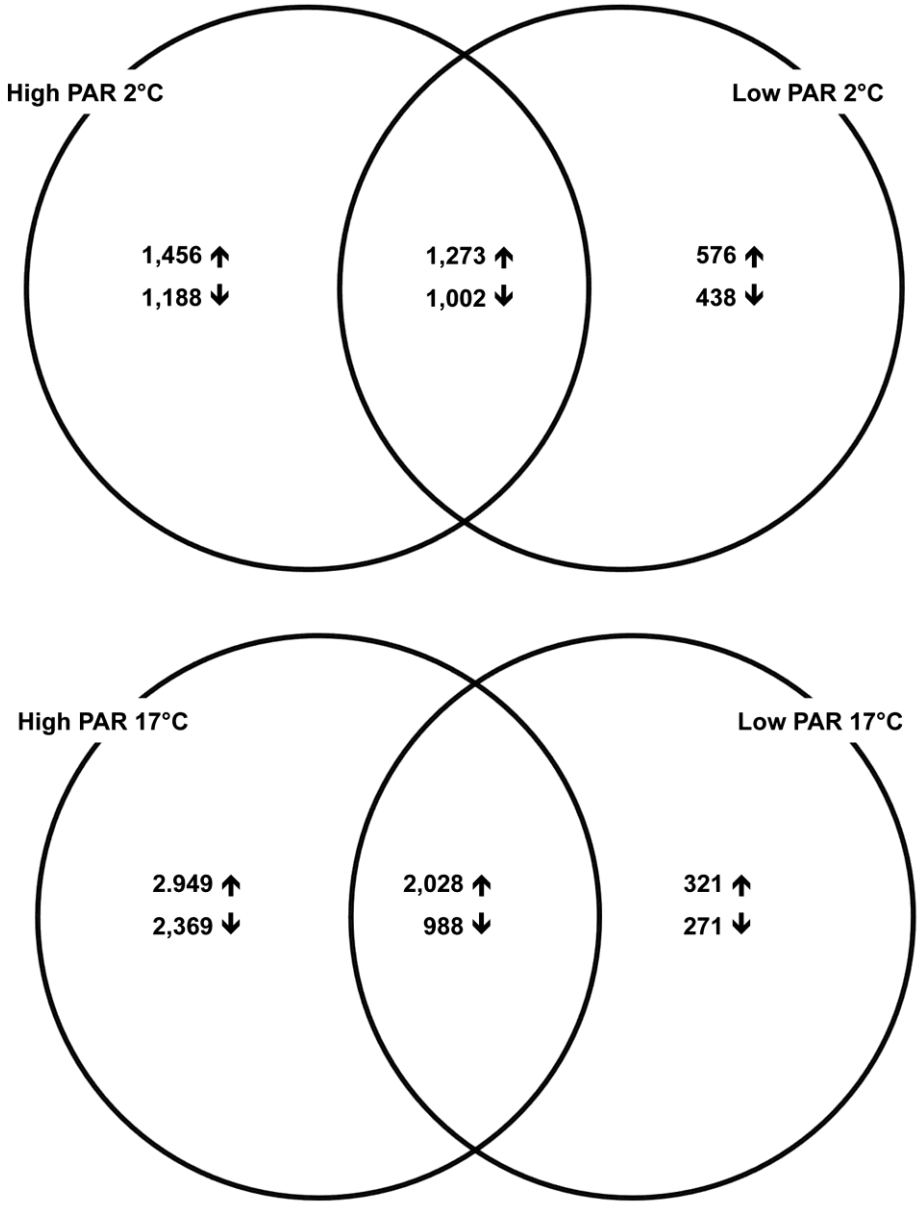


Figure 3. Venn diagram of responsive ESTs after 24h exposure to different light and temperature conditions.



Publication III

(submitted)

Plant Physiology

Interactive effects of temperature, high photosynthetically active radiation, and UV radiation on gene expression in *Saccharina latissima* (Phaeophyceae)

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Running head:

Effects of abiotic stress on gene expression in kelp

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Interactive effects of temperature, high photosynthetically active radiation, and UV radiation on gene expression in *Saccharina latissima* (Phaeophyceae)

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ABSTRACT

Macroalgae of the order Laminariales (kelp) are important components of cold-temperate coastal ecosystem. Important factors influencing their distribution are light including UV radiation and temperature therefore future global environmental changes could potentially have a significant impact on their zonation, distribution patterns, and primary productivity.

Even though many physiological studies were performed on UV radiation and temperature stress in kelp, so far no study is available on the molecular processes involved in acclimation to these stresses. Therefore sporophytes of *S. latissima* were exposed for two weeks to 12 different combinations of photosynthetically active radiation, UV radiation and temperature. Subsequently microarray hybridizations were performed to determine changes in gene expression patterns. Several effects on the transcriptome were observed after the exposure experiments. Strongest effect of temperature on gene expression was observed at 2°C. Furthermore UV radiation had stronger effects on gene expression compared to high photosynthetically active radiation, and caused stronger induction genes correlated to several categories, e.g. photosynthetic components and vitamin B_6 biosynthesis. Higher temperatures seem to ameliorate the negative effects of UV radiation in *S. latissima*. A striking feature was the sophisticated regulation of ROS scavengers, functioning in compartment specific ROS scavenging mechanisms. Gene expression profiles of ROS scavengers indicate a high amount of photo-oxidative stress in response to the 2°C condition as well as mitochondrial ROS formation in response to excessive light at 12°C.

INTRODUCTION

Marine Macroalgae are crucial components of coastal ecosystem in terms of biomass and biodiversity and they account for up to 10% of the global oceanic primary production (Smith, 1981; Charpy-Roubaud, 1990). Macroalgae of the order Laminariales (= "kelp") form huge beds, which are structurally complex and highly productive components of cold water rocky marine coastlines (Steneck et al., 2002). Furthermore they comprise a marine resource for food, alginic acid, biochemicals and pharmaceuticals (Crépineau et al., 2000; Waaland et al., 2004; Roeder et al., 2005). Whereas the geographical distribution of kelps is determined by temperature demands for growth and reproduction, as well as by temperature tolerance of the different life-cycle stages (tom Dieck, 1992; Wiencke et al., 1994; Müller et al., 2008), their vertical distribution is determined by the abiotic factor light, including UV radiation (Hanelt, 1998; Roleda et al., 2005; Wiencke et al., 2006).

In recent years strong decreases of stratospheric ozone density, especially in the polar regions, have been reported. A recent study demonstrated that the chemical ozone destruction over the Arctic in early 2011 was comparable to that in the Antarctic ozone hole (Manney et al., 2011). Stratospheric ozone depletion leads to an increase of UV-B radiation at the earth`s surface (Michler et al., 2002; Franklin et al., 2003). In the worst case there will be an ozone depletion over the Arctic of up to 20 % until 2020 (WMO, 2006). UV radiation (UVR) is absorbed by nucleic acids, proteins and lipids, consequently it is potentially damaging to biological systems (Vass et al., 2005; Bischof et al., 2006; Davison et al., 2007). Like other abiotic stresses, UVR leads to the formation of reactive oxygen species (ROS), which cause cellular damage by denaturation of, e.g. proteins, nucleic acids, and lipids (Dring, 2006; Lesser, 2006). A large number of physiological studies have been conducted in macroalgae on acclimation to UV-stress (Karsten et al., 2011). UVR negatively affects maximum quantum yield of photosystem II and the electron transport rate (ETRmax) of macroalgae (Dring et al., 1996; Hanelt et al., 1997; Bischof et al., 1998). Subsequent studies demonstrated that UVR leads to degradation of the light-harvesting complex (LHC) and the photosystem II D1 protein, recovery mechanisms after excess light include degradation of damaged D1 proteins and it`s de novo synthesis (Bischof et al., 1998; Wiencke et al., 2000; Karsten et al., 2001; Franklin et al., 2003; Davison et al., 2007). Furthermore it was shown, that UVR negatively affects secondary photosynthetic reactions (Bischof et al., 2000; Bischof et al., 2002). Apart from the effects on photosynthesis, UVR also causes DNA damage by disturbing normal base pairing of DNA, which leads to the formation of cyclobutane-pyrimidine dimers (CPDs) (van de Poll

et al., 2001). These dimers inhibit genome replication and gene expression, causing disruption in cell metabolism and division (Buma et al, 1995, Roleda et al., 2005; Roleda et al., 2006). It is likely that future ozone depletion and the resulting enhancements of UVR will influence zonation, distribution patterns, and primary productivity of kelp at their present habitats.

Kelp communities are not only threatened by enhanced UVR, but also by global warming. In the past century overall global warming was around 0.8°C (Hansen et al., 2006). For the Arctic, a similar warming trend has been observed over recent decades. Monthly temperature anomalies can be as large as 3-4°C (Turner et al., 2007). In cold- and warm-temperate localities the reported mean annual temperature increase was as high as >2°C depending on location and duration of time series within the last decades (e.g., Portugal: Lima et al., 2007 North Sea: Wiltshire et al., 2008)

Even though many physiological studies were performed on UV and temperature stress in kelp, until now no study is available on the molecular processes involved in acclimation to these stresses. Thus the aim of this study was to determine changes in gene expression in response to different combinations of photosynthetically active radiation (PAR), UVR and temperature in a kelp species. The object of our studies was the perennial kelp *Saccharina latissima*, which is a common species in kelp beds of polar and temperate coastal waters and occurs from the intertidal till 30m depth (Bolton et al., 1983; Gerard, 1988; Borum et al., 2002). Sporophytes of this species were exposed for two weeks to different combinations of photosynthetically active radiation and UVR at three different temperatures (2,7,12°C). Maximum quantum yield of photosystem II was determined twice a week during the experimental duration for observing the extent of photoinhibition. For investigating molecular mechanisms of acclimation to high photosynthetically active radiation, and UVR gene expression profiles were assessed through microarray hybridizations.

RESULTS

Photosynthetic efficiency

Our results demonstrate the interactive effects of the combined stressors high PAR, UV radiation, and temperature. The mean maximum quantum yield of photosystem II (Fv/Fm) of sporophytes of *S. latissima* under combinations of radiation and temperature conditions over a time course of 14 days is shown in Fig. 1. Maximum quantum yield (Fv/Fm) remained constant over the time at low PAR (LP) as well as under low PAR + UV (LPAB) under the

three tested temperature regimes, and was significantly higher than under high PAR (HP) and high PAR + UV conditions (HPAB) ($p < 0.0001$), furthermore maximum quantum yield was significantly lower after exposure to HPAB than to HP under the three tested temperatures ($p < 0.0001$). Initial mean maximum quantum yield (Fv/Fm) was 0.633 ± 0.02 (day 0). At day 3 of exposure a significant decrease in Fv/Fm was observed in sporophytes exposed to HP and HPAB conditions at all tested temperatures, with the strongest inhibition occurring under the HPAB treatment at 2°C (Fv/Fm of 0.24 ± 0.05). Additionally, at both radiation conditions, the decrease of the maximum quantum yield of photosystem II was significantly higher at 2°C compared to 7°C and 12°C ($p < 0.05$). From day 3 to day 7 the Fv/Fm values increased at HP and HPAB conditions at all experimental temperatures, with a significantly lower increase at 2°C than at 7°C for both conditions ($p < 0.05$). At the end of the experiment (day 14) we observed a significant higher *Fv/F*m at 2°C HP (Fv/Fm= 0.569 ± 0.04) than at 2°C HPAB (Fv/Fm= 0.491 ± 0.002); at 7°C und 12°C no significant difference between the HP and HPAB treatment was detectable. The only interactive effect of temperature and light at day 14 was observed within the HPAB treatment, where the maximum quantum yield of photosystem II was significantly lower ($p < 0.05$) at 12°C (Fv/Fm= 0.445 ± 0.03) than at 2°C (Fv/Fm= 0.491 ± 0.002) and at 7°C (Fv/Fm= 0.543 ± 0.03), respectively.

Gene expression profiles

Gene expression profiles under temperature, high PAR, and UVR stress were determined using oligonucleotide microarrays covering 25,262 transcripts. 6,563 transcripts (26%) showed different expression patterns under at least one stress treatment compared to the control treatment (2°C/7°C/12°C & low PAR). The strongest effect on gene expression was observed in the 2°C HPAB treatment when 2,228 genes (9%) showed a significant different expression compared to the control, followed by the 12°C HPAB and the 12°C HP condition, at which 2057 (8%) and 2022 (8%) genes, respectively, were effected. The lowest amount of regulated genes occurred in response the 7°C LPAB, when 397 genes (2%) showed an altered expression. The remaining five treatments (2°C/7°C HP, 7°C HPAB; 2°C/12°C LP) caused transcriptional changes for 547 – 1,559 (2-6%) of the genes.

Gene Ontology enrichments (FDR < 0.05) were performed for all significantly up- and down-regulated genes under the different stress conditions (Table I). We detected 106 enriched GO terms with the following distribution 55, 42, and 9 enriched GO terms at 2°, 7°, an 12°C treatments, respectively. The highest number of over-represented GO terms in a single

treatment was found within the up-regulated genes under 2°C HPAB conditions followed by the 7°C LPAB treatment, here we found 35 respectively 23 enriched GO terms. For a detailed list see table S1. In general the number of enriched GO terms increased with decreasing temperature and under UV light. Among the induced genes at 2°C high PAR the GO terms plastid (GO:0006519), chloroplast (GO:0009507), and rRNA binding (GO:0019843) were identified as being over-represented, whereas the terms peroxidase activity (GO:0019843), oxidoreductase activity, acting on peroxide as acceptor (GO:0016684), and antioxidant activity (GO:0016209) were enriched among the down-regulated genes at 2°C high PAR. Both UV treatments (LPAB/HPAB) at 2°C led to an enhanced up-regulation of genes associated with photosynthetic components (e.g. thylakoid part GO:0044436, photosynthetic membrane GO:0034357), and metallopeptidase activity (GO: 0008237). Within the category biological process the 2°C HPAB treatment showed a higher induction of GO terms correlated to cellular amine metabolic processes such as cellular amino acid metabolic process (GO:0006520), and aromatic amino acid family biosynthetic process (GO:0009073), as well as to vitamin metabolic processes (e.g. water-soluble vitamin biosynthetic process GO:0042364, vitamin B_6 metabolic process GO:0042816). Among the up-regulated genes of the 2°C LPAB treatment GO terms correlated to photosynthesis (GO: 0015979), and vitamin metabolic process (GO:0006766) occurred at higher frequency than expected statistically.

High PAR conditions at 7°C led to an enhanced up-regulation of genes correlated to carbohydrate binding (GO:0030246), and glycine metabolic process (GO:0006544), including glycine catabolic process (GO:0006546), as well as glycine decarboxylation via glycine cleavage system (GO:0019464). Among the induced genes at 7°C LPAB and 7°C HPAB we detected 23, and 10 respectively, enriched GO terms correlated to photosynthetic components. The 7°C HPAB responsive ESTs featured furthermore enriched GO terms connected to vitamin metabolic process (vitamin B_6 metabolic process GO:0042816), glycine metabolic process (glycine decarboxylation via glycine cleavage system GO:0019464), and photosynthesis (GO: 0015979). Up-regulated genes at 7°C LPAB showed additionally a higher abundance of the GO terms electron transporter, transferring electrons within the cyclic electron transport pathway of photosynthesis activity (GO:0045156), and photosynthetic electron transport in photosystem II (GO:0009772).

Induced transcripts at 12°C HP/HPAB showed an enrichment of the GO terms peroxidase activity (GO:0019843), oxidoreductase activity acting on peroxide as acceptor (GO:0016684), and antioxidant activity (GO:0016209). Among the repressed transcripts at 12°C/HP the GO term photosynthesis (GO: 0015979) was found to be over-represented. The 12°C HPAB

condition led additionally to an enhanced up-regulation of genes associated with the GO terms chloroplast (GO: 0009507), and plastid (GO:0009536).

Metabolic pathway enrichments

We identified 5 different significantly enriched pathways among the up- and down regulated genes of the different stress treatments (Table II). Exposure to 2°C HPAB and 7°C HP resulted in an induction of genes involved in glycine, serine and threonine metabolism. Among the up-regulated genes under 2°C LPAB we observed an increased regulation of the carotenoid biosynthesis. The 7°C HPAB treatment featured down-regulation of arachidonic acid metabolism and the PPAR signalling pathway. A striking feature was the enhanced up-regulation of the vitamin B_6 metabolism, which occurred under HPAB conditions at 2°C, 7°C and 12°C, as well as after exposure to 2°C HP.

Manual inspection of potential high PAR and UV responsive genes

To investigate the molecular mechanisms of acclimation of photosynthesis to high levels of photosynthetically active radiation and UV under different temperature regimes, we analysed manually transcriptional changes of genes encoding for photosynthetic components and chlorophyll metabolism (Table III). Because KEGG pathway enrichments indicated that metabolic adjustments in several treatments includes regulation of vitamin B_6 metabolism, we examined the expression profiles of genes involved in these pathway (Table IV). Furthermore we inspected the expression of reactive oxygen species (ROS) scavenging enzymes (table IV), as reactive oxygen species being a known abiotic stressor. A full list of the regulated genes with annotations can be retrieved from the supplemental material (table S2).

We observed significant up-regulation of several transcripts encoding photosynthetic components in response to the stress treatments, but no down-regulation within this category. The highest number of responsive transcripts was found in the 2°C HPAB treatment, were all genes, except one, were found to be upregulated, followed by the 2°C LPAB and 7°C HPAB condition, where 11 out of 14 transcripts were induced. The strongest up-regulation of up to 18-fold occurred in genes correlated to the light-harvesting complex (contig13579, contig01362). We detected three transcripts encoding for photosystem I, photosystem I assembly protein ycf4 (contig04207), photosystem I p700 chlorophyll a apoprotein a1 (contig02615) and photosystem I reaction centre subunit II, which were mostly induced after the exposure treatments at 2°C. We observed 6 regulated transcripts correlated to photosystem II with transcriptional changes between 2 – 2.8-fold, none of them were regulated in response

to the HP treatments. For example photosystem II reaction centre protein D1/psbA (contig01925) and photosystem II reaction centre protein y (contig13305) were induced after all HPAB treatments and 2°C /7°C LPAB, photosystem II D2 protein (contig03429) 7°C/12°C HPAB and 7°C LPAB. Transcripts encoding for the cytochrome b6 complex showed higher abundance at 2°C and 7°C HPAB/LPAB. Furthermore we observed induction of the chloroplastic thylakoid lumenal 15 kDa protein (contig15193) mainly in response to the HPAB treatments, whereas thylakoid lumen peptidyl-prolyl cis-trans isomerase 40 kDa (contig03384) was mostly up-regulated in response to the LPAB conditions.

Within the porphyrin and chlorophyll metabolism, magnesium chelatase subunit h (contig27613) was induced at 2°C/7°C HPAB and 2°C LPAB, whereas coproporphyrinoegen II oxidase (contig18633) and pheophorbide a oxygenase (contig16043) showed higher transcript abundance at 2°C and 7°C HPAB. The geranylgeranyl diphosphate reductase (contig06100) was induced after all 2°C treatments and 7°C HPAB, but repressed at 12°C HP. A striking feature was the significant up-regulation of transcripts correlated to vitamin B_6 metabolism in response to the HPAB treatments, with the highest induction after the 2°C HPAB condition. Additionally, pyridoxal 5-phosphate synthase (contig03286) was up-regulated after exposure to LPAB at 2°C/7°C/12°C, pyridoxal biosynthesis protein pdx1 (contig09890) was induced in response to HP at 2°C and 7°C, whereas pyridoxal biosynthesis protein pdx1.3 (contig20055) showed higher transcript abundance in response to 2°C LPAB and 7°C HP. Furthermore we observed up-regulation of pyridoxal biosynthesis protein pdx2 (contig05224) after the 2°C LPAB exposure.

Several genes encoding for antioxidative enzymes were significantly induced. Ascorbate peroxidase (contig2732) and superoxide dismutase [Mn] (contig12014) showed a higher transcript abundance at 12°C HP/HPAB, whereas superoxide dismutase [Fe] (contig04061) was induced in response to 2°C HP and 7/12°C HPAB. We observed induction of glutathione reductase (contig03562) after the 2°C/7°C HPAB and 2°C LPAB treatments. Furthermore we detected three transcripts correlated to the thioredoxin system, chloroplastic thioredoxin showed a higher transcript abundance after exposure to 2°C HPAB/LPAB, thioredoxin h was induced in response to 12°C HP/HPAB, whereas thioredoxin reductase was up-regulated at 2°C HP and 7°C HPAB conditions.

DISCUSSION

Photosynthetic responses to changed radiation and temperature conditions

Maximum quantum yield (Fv/Fm) remained stable at low photosynthetically active radiation (PAR) as well as under low PAR + UV under all tested temperatures, while sporophytes exposed to high PAR and high PAR + UV showed a significant decrease in the maximum quantum yield of PS II, with the highest degree of photoinhibition occurring after the high PAR + UV treatment. In the first week inhibitory effects were stronger at high PAR + UV than at high PAR alone. However, at the end of the experiment no significant differences in Fv/Fm between these treatments were detected for 7°C and 12°C; at 2°C maximum quantum yield was significantly higher after the high PAR treatment. Considering the fact, that UV under low PAR conditions did not influence maximum quantum yield of PS II, we suggest that the observed photoinhibitory effects are mostly caused by high PAR. This is consistent with other studies on macroalgae, where it was shown that photoinhibition in several Arctic macroalgae was mainly induced by white light, and that UV radiation caused a delay in recovery (Hanelt et al., 1997; Bischof et al., 1999; Dring et al., 2001). The rate of photoinhibition decreased due to acclimation over the course of the experiment; at the end of the experiment high PAR and high PAR + UV treatments featured average recovery rates of maximum quantum yield of 87% respectively 78%, compared to the low PAR and low PAR + UV treatments. These results are in agreement with other studies on brown algae, where during the process of acclimation of photosynthetic activity the recovery rate from UV-induced photoinhibition increased (Bischof et al., 1998; Bischof et al., 1999).

In our study genes for several components of both photosystems featured up-regulation, mostly in response to UV treatments at 2°C and 7°C, within these treatments we also observed the highest number of enriched GO terms correlated to photosynthetic components among the induced genes. The lower induction rates of transcripts at 12°C compared to 2°C indicates an ameliorating effect of increasing temperature, even though no significant differences in maximum quantum yield were detected. All components of the photosystems, except the photosystem I p700 chlorophyll a apoprotein a1, were solely induced in response to the UV treatments, suggesting that UV radiation rather than high PAR led to an enhanced turnover of proteins related to the photosystems. Transcripts encoding for photosystem II reaction centre protein y and photosystem II reaction centre protein D1/psbA were induced in response to all UV-treatments, except at 12°C low PAR + UV. Studies on the effect of UV radiation in the cyanobacteria revealed repair of UV damaged PS II reaction centre by

increasing the transcript pool, the accumulation of these transcripts then leads to a gradual replacement of the damaged D1 reaction centre (Sass et al., 1997; Campbell et al., 1998; Máté et al., 1998; Huang et al., 2002). Our results indicate that the molecular mechanism responsible for the repair of UV-induced photodamage is similar to the one discovered in cyanobacteria. Photosystem II showed a higher susceptibility towards UV radiation than photosystem I, which was reflected by the higher number of regulated transcripts associated with PS II, as well as the higher number of treatments, which featured induction. This was also demonstrated in previous studies, which showed that the PS II is the primary target of UV-B radiation (Huang et al., 2002; Franklin et al., 2003; Vass et al., 2005).

We detected induction of genes encoding for light-harvesting complex proteins as well as porphyrin and chlorophyll metabolism. Previous studies on pigment contents in macroalgae under stress showed ambivalent results, higher pigment content in response to UV radiation was measured in the green alga *Ulva rigida* (Altamirano et al., 2000) and the red alga *Palmaria decipiens* (Poppe et al., 2002), whereas *Laminaria* species showed no significant changes in chlorophyll a concentration after exposure to UV radiation (Roleda et al., 2006). For green plants it was shown that the function of LHCs is not only to harvest light through chlorophyll excitation and to transfer energy to the reaction centre, but also to prevent damage to the photosystem by quenching potentially harmful chlorophyll triplets (Carbonera et al., 2005; Standfuss et al., 2005; Mozzo et al., 2008). The observed induction of light-harvesting complex proteins in response to light stress indicates a role in photo protection. It appears that this process is a component of the long-term acclimation of photosynthesis to both, high PAR and UV radiation, in *S.latissima*.

We observed the up-regulation of transcripts correlated to chlorophyll biosynthesis, e.g. coproporphyrinogen III oxidase and magnesium-chelatase subunit mainly in response to the 2° and 7°C HPAB treatments. In parallel with the induction of chlorophyll biosynthesis, enhanced expression of pheophorbide a oxygenase was detected after the 2° and 7°C HPAB treatments. Pheophorbide a oxygenase is a key control point in the overall regulation of chlorophyll degradation and is responsible for catalysing the oxygenic ring opening of pheophorbide (Chung et al., 2006; Hirashima et al., 2009). The data suggests that severe damage on the light harvesting complex did occur under high PAR + UV conditions at 2°C and 7°C, leading to enhanced degradation of chlorophyll molecules. Nevertheless our finding of only slightly reduced maximum quantum yield of PS II of these treatments compared to the high PAR conditions indicates that *S. latissima* was able to compensate most of the degradation of LHCs by transcriptional regulation.

In summary our gene expression results support previous physiological studies in macroalgae, which showed that effects of UV radiation on photosynthesis include e.g. degradation of the light-harvesting complex (LHC) and the photosystem II D1 protein, recovery mechanism after excess light include degradation of damaged D1 proteins and the de novo synthesis (Bischof et al., 1998; Wiencke et al., 2000; Karsten et al., 2001; Davison et al., 2007).

Metabolic adjustments in response to high PAR and UV stress

We observed significant changes in gene expression; 26% of the studied genes exhibited transcriptional changes under at least one stress treatment compared to the control treatment. The highest amount of regulated genes was detected in response to high PAR + UV at 2°C, where 2,228 genes (9%) showed a significantly different expression compared to the control, indicating that acclimating to this condition requires the highest amount of metabolic reorganization. Whereas under low PAR + UV conditions most of the enriched GO terms were correlated to photosynthesis, high PAR + UV treatments featured several enriched GO terms related to other metabolic pathways. High PAR + UV at 2°C and 7°C as well as 7°C high PAR led to an enhanced induction of transcripts correlated to cellular amino acid metabolism, at this the highest amount of GO terms related to this category was found after exposure 2°C high PAR + UV. KOBAS metabolic pathway enrichments showed similar results. We detected enhanced up-regulation of glycine, serine and threonine metabolism at 2° high PAR + UV and 7°C high PAR. These results indicate that the regulation of glycine, serine and threonine metabolism plays an important role in acclimation to these conditions. Until now no study is available on the influence of abiotic stress on amino acid metabolism in macroalgae. However, serine is involved in the final step of cysteine biosynthesis and cysteine, glycine and glutamate are essential for synthesis of glutathione (GSH), which acts as a reducing co-factor for several enzymes involved in reactive oxygen species (ROS) detoxification (Noctor et al., 1997; Noji et al., 1998). The induction of transcripts of the glycine, serine and threonine metabolism might caused by an increased production of ROS during exposure to high PAR + UV at 2°C and 7°C.

Carotenoid metabolism was found to be enhanced induced at both UV treatments at 2°C. Carotenoids constitute important components of the light-harvesting complex, where they have a dual function in light harvesting and photo-protection, as they scavenge singlet oxygen and trap triplet states of chlorophyll (Horton and Ruban, 2005; Standfuss et al., 2005). In green plants carotenoids have a protective function against UV radiation (Campos et al., 1991; Rau et al., 1991; Middleton and Teramura, 1993). Studies in cyanobacteria indicated

that carotenoids might be involved in inactivating UV-B induced radicals in photosynthetic membranes (Woodall et al., 1997; Götz et al., 1999). We therefore consider that the induction of the carotenoid metabolism might be connected on one hand to the observed enhanced synthesis of light harvesting complexes in these treatments, and on the other hand to enhanced appearance of UV-induced radicals at 2°C.

Gene enrichment and KEGG metabolic pathway enrichments revealed strong up-regulation of transcripts correlated to vitamin B_6 metabolism under the combined stressors high PAR and UV radiation. Vitamin B_6 is a water-soluble vitamin, which acts as a cofactor for a large number of essential enzymes, mostly associated with amino acid synthesis (González et al., 2007; Leuendorf et al., 2010; Mooney and Hellmann, 2010). Vitamin B_6 exhibits a antioxidant activity comparable to that of vitamins C and E, for this reason it is suggested to play an important role in protecting cells from oxidative stress (Ehrenshaft et al., 1999). Studies on *Arabidopsis* showed that vitamin B_6 is linked to stress responses, it is crucial for acclimation to oxidative and osmotic stress (Chen and Xiong, 2005), in addition it seems to function in photo protection (Titiz et al., 2006). The induction of vitamin B_6 metabolism seems to be a critical component of the molecular acclimation mechanisms to excessive light in *Saccharina latissima*, and might enable preventing damage by oxidative stress.

Induction of ROS defence

The rapid formation of ROS is a well-known component of UV-B stress, and in general, of increased physiological stress conditions (Hectors et al., 2007; Kumar et al., 2010). ROS, such as superoxide and hydrogen peroxide, cause cellular damage in terms of denaturation of nucleic acids, proteins, polysaccharides and lipids (Contreras et al., 2009; Lehmann et al., 2009; Kumar et al., 2010). Plants are able to counter ROS by a sophisticated network of non-enzymatic and enzymatic systems, which scavenge the various ROS intermediates (Asada, 1997, 1999; Foyer and Noctor, 2005). We detected significant induction of several transcripts correlated with reactive oxygen species (ROS) scavenging mechanisms. Ascorbate peroxidase, thioredoxin h and superoxide dismutase [Mn] showed a higher transcript abundance after exposure to high PAR and high PAR + UV conditions at 12°C, whereas superoxide dismutase [Fe] was induced in response to 2°C HP and 7°C/12°C HPAB. Chloroplastic thioredoxin, thioredoxin reductase and glutathione reductase were mainly up-regulated in response to the 2°C conditions and/or in response to high PAR + UV at 7°C.

Superoxide dismutase (SOD) catalyses the reaction of superoxide anion to hydrogen peroxide and oxygen (Apel and Hirt, 2004). It occurs as different metalloproteins (Fe, Mn, CuZn, and

Ni) with different cellular distributions, Fe SOD is principally located in chloroplasts, whereas Mn SOD is located in mitochondria (Wolfe-Simon et al., 2005; Lesser, 2006). Studies on *Nicotiana plumbaginifolia* showed, that Fe SOD responds to increased ROS formation in chloroplasts, while Mn SOD responds to increased oxyradical formation in the mitochondria, it is therefore suggested that expression of a particular SOD form is likely to be governed by the cellular location, where oxidative stress is generated (Bowler et al., 1989; Tsang et al., 1991; Bowler et al., 1992). Ascorbate peroxidase and glutathione reductase are detoxifying hydrogen peroxide in the ascorbate-glutathione cycle, which is considered to play an important role in ROS scavenging (Asada, 1997; Noctor, 1998; Asada, 1999). In green plants key components of the ascorbate-glutathione cycle are localized in several cellular compartments, e.g. chloroplasts and mitochondria (Mittler, 2002). In *Arabidopsis* these endogenous ascorbate and glutathione-based antioxidant systems are encoded by single organelle targeted isoforms (Mittova et al., 2000; Chew et al., 2003). Plants contain several isoforms of Trx in several subcellular compartments, e.g. chloroplasts, mitochondria and cytosol (Schürmann and Jacquot, 2000; Jaquot, 2009). Chloroplast Trx are induced in response to high light intensities, they are regulating via ferredoxin-thioredoxin reductase photosynthetic enzymes by light. Furthermore they are critical for redox regulation of protein function and signalling via thio redox control, and are supposed to be involved in redox balancing related to photosynthesis (Arner and Holmgren, 2000; Vieira Dos Santosa, 2006). Trx h is located in mitochondria and cytosol, it has been proposed to be involved in cellular protection against oxidative stress as well as modulating redox-dependent signalling cascades (Gelhaye et al., 2004; Jaquot, 2009).

The two main expression patterns of the different transcripts involved in ROS scavenging in our study, either induced at 2° or 12°C, might be due to the subcellular location of the encoded gene products and possibly reflects changes in ROS levels in chloroplasts and mitochondria. The induction of Mn SOD at 12°C indicates an increased ROS evolution in mitochondria. The similar expression pattern of Mn SOD, ascorbate peroxidase, and thioredoxin suggests that all these transcripts are involved in mitochondrial ROS detoxification. The rate of mitochondrial superoxide generation is altered by physiological conditions; increased temperature can lead to impairments in mitochondrial functions and results in an induction of oxidative damage (Turrens, 1997; Vacca et al., 2004; Larkindale et al., 2005). One major mechanism of ROS production in mitochondria is the over-reduction of components within the mitochondrial electron-transport chain (Davidson and Schiestl, 2001; Møller et al., 2007). Plant mitochondria have been overlooked for a long time as source of

ROS, maybe because estimates suggest that formation of mitochondrial ROS is considerably lesser than that of chloroplast and peroxisomes in light (Sweetlove et al., 2002; Foyer and Noctor, 2003). Nevertheless, subsequent studies showed that changes in mitochondrial ROS production have consequences for the cell, e.g. peroxidation of the mitochondrial membrane as well as damage and inhibition of mitochondrial proteins (Rhoads et al., 2006; Noctor et al., 2007; Schwarzlander et al., 2009). The enhanced transcript abundance for chloroplastic thioredoxin, thioredoxin reductase and glutathione reductase under high irradiance at 2°C might be correlated to enhanced photo-oxidative stress. Under excessive light photo-oxidative stress arises from an over-reduction of the photosynthetic electron transport chain, low temperature leads to an additional increase of photo-oxidative stress, which is partly caused by a reduced activity of the Calvin cycle (Pfannschmidt, 2003; Haghjou et al., 2009). Taken together, our findings indicate the existence of compartment specific ROS scavenging mechanisms in *Saccharina latissima.* Expression profiles of ROS scavengers indicate a high amount of photo-oxidative stress in response to the 2°C condition as well as mitochondrial ROS formation in response to excessive light at 12°C.

CONCLUSION

In our study we investigated the concerted action of molecular mechanisms underlying physiological acclimation to light and UV-stress in *Saccharina latissima*. Our results provide an initial idea of how complex the molecular basis of acclimation is. The effect of stress on the transcriptional level was stronger than on the physiological level. Gene expression profiles under low PAR and UV showed, that a certain amount of stress can be compensated for by transcriptional regulation, without observable effect on the efficiency of PS II. The exposure to the additional stressor high PAR seems to exceed the "stress capacity" of the system. Strongest effect of temperature on gene expression was observed at 2°C, furthermore UVR had stronger effects on gene expression than high PAR, and led to stronger induction of genes associated to several categories, e.g. photosynthetic components and vitamin B_6 biosynthesis. UVR caused high synthesis rates of the photosynthetic reaction centre. We conclude that UV radiation leads to severe damage of photosynthetic reaction centres, of which photosystem II was more susceptible to UV-stress than photosystem I.

The favouring of transcripts of UVR stress response, and the associated energy demands for repair and protection, might divert products of photosynthesis at the expense of growth, and on longer time scales might lead to reduced growth rates, especially at 2°C. Higher

temperatures seem to ameliorate the negative effects of UVR in culture material of *S. latissima*. However, for predicting biological effects of changing abiotic factors on *S. latissima* in the field, it is necessary to conduct studies with field material, for investigating whether origin, and therefore "life story" of the algae, influences gene expression profiles under abiotic stress.

MATERIAL AND METHODS

Algal material

Sporophytes of *Saccharina latissima* (Lane et al., 2006) were raised from gametophytes cultures, established from spores of fertile sporophytes collected by SCUBA diving in Kongsfjorden (79°N; 11°E; Svalbard, Norway, AWI culture numbers: 3123, 3124). Male and female gametophytes were fragmented together, transferred to petri dishes filled with Provasoli enriched seawater (PES) (Starr and Zeikus, 1993) and cultured at 10 +/- 1°C and 30 µmol photons m^{-2} s^{-1} white light at 18h light: 6h dark period. After 2 weeks developing sporophytes were transferred to aerated 5l culture bottles and grown in PES until they reached a size of 5-7cm. To exclude thalli contaminated by endophytes, cultures were examined by microscopy.

Irradiance & temperature experiments

Young sporophytes were exposed for 14 days in environmentally controlled rooms (2, 7 and 12°C ± 1°C) to high photosynthetically active radiation (PAR) and low PAR in combination with UV radiation (UVR), which was supplemented daily in the middle of the light-phase (0900–1500 H). Experimental light conditions are shown in table V. All experiments were conducted in 5 replicates. To distinguish the effects of different wavelengths ranges, the experimental units were covered with cut-off filter foils permeable to wavelengths: (1) 400-700 nm (URUV Ultraphan UV farblos, Difrega, Germany) for photosynthetically active radiation (PAR) treatments, and (2) 295-700nm (URT 140 Ultraphan UV farblos, Difrega) for PAR + UVA + UVB treatments. PAR was provided by 3/5 Osram daylight fluorescent tubes (Biolux, 36W; Osram, Germany) and measured by using a LI- 250 light meter (LI-COR, Lincoln; USA). UV irradiance was generated by 3 fluorescent tubes (UV A-340, 40W; Q-Panel, USA) and determined with a Solar Light PMA-2100 (Solar Light; PA, USA).

Fluorescence measurements

For observing the extent of photoinhibition during the experimental duration of 2 weeks, photosynthetic efficiency was measured twice a week. Sporophytes were dark-adapted for 5 min prior to the measurements. Afterwards the maximum quantum yield of PS II (*F*v/*F*m) was measured with an Imaging PAM (Pulse Amplitude Fluorometer, Walz, Effeltrich, Germany). After the fluorescence measurements the sporophytes were frozen in liquid nitrogen and stored at -80°C until further use. Fv/Fm values of sporophytes obtained under the different conditions were analysed using a two-way ANOVA with repeated measurements ($p < 0.05$). Significant differences and interaction of means were compared with the post hoc Tukey test (HSD, $p < 0.05$). All statistical analyses were done using SPSS software version 19 (IBM, USA).

RNA-extraction and -labelling

Total RNA was extracted according to (Heinrich et al., 2011). For total RNA labelling the Agilent two-colour Low Input Quick Amp Labeling kit (Agilent Technologies, Waldbronn, Germany) was used. RNA from stress treatments was labelled by fluorescent complementary RNA (cRNA) synthesis with cyanine-5-CTP, control treatment RNA was labelled with cyanine-3-CTP.

Prior to the labelling, the Agilent RNA Spike-In Mix (Agilent) was added to 200 ng of total RNA. Due to the extensive length of 3`untranslated regions (UTRs) occurring in brown algae cDNA synthesis was performed using a blend of T7 promoter primer and T7 nonamer primer in equal molarity. cRNA synthesis and purification of labelled RNA was performed according to the two-colour Low Input Quick Amp Labelling kit protocol (Agilent). Specific activity of cyanine-3 and cyanine-5 and cRNA yield was determined using the NanoDrop ND-100 spectrometer (PeqLab, Erlangen, Germany).

Microarray design and hybridization

6x80 k microarrays slides were designed with Agilent's eArray online application tool containing 60mer oligonucleotides probes, which were designed from a *Saccharina latissima* cDNA library, thereby 25,262 transcripts were represented by either 1, 2, or 3 individual probes. The cDNA library was established from RNA sampled under various light and temperature regimes and exhibits functional genome coverage of approximately 70%, (Heinrich et al., 2011). Original sequencing files of the cDNA library were uploaded at the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra; accession number

SRR305166). Sequences of the cDNA library were annotated with Blast2GO (Conesa et al., 2005) and blasted against the Swiss-Prot protein knowledgebase (http://www.uniprot.org/) (release 2010_7) and the NCBI non-redundant protein database (http://blast.ncbi.nlm.nih.gov/Blast.cgi, NCBI-nr using the BLASTX algorithm with an E-value cut-off of 10^{-7}.

Total RNA of the different treatments was hybridized against a pooled control consisting of RNA from all low PAR treatments (2°C/7°C/12°C). Hybridizations were carried out in 4 replicates and were performed with 300ng of cyanine-3 and cyanine-5 labelled cRNA for 17h at 65°C. Afterwards microarray disassembly and wash procedure followed according to manufacturer's instructions (Agilent). Microarrays were scanned with the Agilent G2565AA scanner. Raw data processing was carried out with the Agilent Feature Extraction Software version 9.1.3.1 (FE), for quality monitoring of the microarrays the Agilent QC Tool (v1.0) with the metric set GE2_QCMT_Feb07 was used. The microarray design, raw data and normalized data as well as the detailed experimental design are MIAME compliant and deposited in a MIAME compliant database (ArrayExpress at the EBI; http://www.ebi.ac.uk/microarray-as/ae/; ID: E-MEXP-3556).

Statistical analysis of microarray data

Changes in gene expression were determined using the GeneSpring GX software platform version 11 (Agilent) with the implemented statistical tests. We performed an ANOVA, followed by a post hoc test Tukey-HSD with the Benjamini Hochberg FDR (false discovery rate) correction. Transcripts were considered to be differentially expressed when p-Values were less than 0.01 and the calculated fold changes between the control and the treatment was at least 2.

Statistical assessments of GO annotations, whose abundance is significantly different between the regulated genes within the various exposure treatments and the whole microarray, were performed by gene set enrichment analysis, which were done using Fisher's exact test within Blast2GO (Conesa et al., 2005),including corrections for multiple testing using FDR, FWER (family-wise error rate) and single test *p*-value. Significantly over-expressed KEGG pathways were identified with KOBAS (http://kobas.cbi.pku.edu.cn/home.do) using a hypergeometric test ($p < 0.01$).

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SUPPLEMENTAL MATERIAL

Table S1

Full list of over-represented Gene Ontology terms within the three GO root categories cellular component, molecular function, and biological process among the different treatments [low photosynthetically active radiation (PAR) treatments = LP; low PAR+UV =LPAB; high PAR = HP, high PAR+UV = HPAB].

Table S2

Full list of regulated ESTs with annotations for all stress conditions [low photosynthetically active radiation (PAR) treatments = LP; low PAR+UV =LPAB; high PAR = HP, high PAR+UV = HPAB].

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FIGURE LEGENDS

Figure 1. Maximum quantum yield of photosystem II (Fv/Fm) of *Saccharina latissima* after exposure at three temperatures (2,7,12°C) and 4 radiation conditions [low photosynthetically active radiation (PAR) treatments = LP; low PAR+UV =LPAB; high PAR = HP, high PAR+UV = HPAB], measured over 14 days of exposure

Figure 2. Total number of differentially up- (black bars) and down-regulated (grey bars) genes after exposure to 3 temperatures (2, 7, 12°C) and 3 radiation conditions: low photosynthetically active radiation + UV (LPAB), high photosynthetically active radiation (HP), and high photosynthetically active radiation + UV (HPAB)

TABLES

Table I. Numbers of over-represented GO terms within the three GO root categories cellular component, molecular function, and biological process among the different treatments [low photosynthetically active radiation (PAR) treatments = LP; low PAR+UV =LPAB; high PAR = HP, high PAR+UV = HPAB].

Temperature	Light condition	Regulation	GO root category		
			Cellular component	Molecular function	Biological process
2	LPAB	up	12	2	2
	HP	up	2	1	-
	HP	down	-	3	-
	HPAB	up	11	2	12
7	LPAB	up	21	1	1
	HP	up	-	1	3
	HPAB	up	10	-	5
12	HP	up	3	-	-
	HP	down	-	-	1
	HPAB	up	2	3	-

Table II. Over-represented metabolic pathways among the significantly up- and down-regulated genes of different stress conditions [low photosynthetically active radiation (PAR) treatments = LP; low PAR+UV =LPAB; high PAR = HP, high PAR+UV = HPAB]. Enriched KEGG pathways were identified with a hypergeometric test ($p < 0.01$).

KEGG pathway	KO Id	Treatment		Regulation
		Temperature	Irradiance	
Carotenoid biosynthesis	ko00906	2°C	LPAB	up
Glycine, serine and threonine metabolism	ko00250	2°C	HPAB	up
		7°C	HP	up
Vitamin B_6 metabolism	ko00750	2°C	HPAB	up
		7°C	HP/ HPAB	up
		12°C	HPAB	up
Arachidonic acid metabolism	ko00860	7°C	HPAB	down
PPAR signalling pathway	ko03320	7°C	HPAB	down

Table III. Differential regulated genes encoding for photosynthetic components, and porphyrin and chlorophyll metabolism. All displayed genes were differentially expressed with $p < 0.01$ and were considered to be significant differently expressed with a fold change > 2 (numbers in bold

Contig name	Putative gene product	Fold change								
		2°C HP	2°C HPAB	2°C LPAB	7°C HP	7°C HPAB	7°C LPAB	12°C HP	12°C HPAB	12°C LPAB
Photosynthetic components										
Contig02465	Cytochrome b6 complex	1.4	**3.1**	**3.5**	1.4	**2.3**	**2.1**	1	1.6	1.1
Contig13579	Fucoxanthin-chlorophyll a-c binding protein	**8.8**	**18.2**	**9.4**	**4.1**	**12.6**	**2**	1	**3**	**1.2**
Contig01362	Light harvesting complex protein	**2.8**	**6.1**	**2.7**	**6**	**4.6**	1.4	**2.5**	**6.7**	-1.4
Contig04207	Photosystem I assembly protein ycf4	1.4	**3.8**	**4.1**	1	1.6	1.8	-1.2	1.5	-1.3
Contig02615	Photosystem I p700 chlorophyll a apoprotein a1	**2.1**	**2.1**	1.5	**2.4**	**2.1**	1.1	1.1	1.6	-1.4
Contig03513	Photosystem I reaction centre subunit II	-1.1	**2.9**	**2.1**	1.2	**2**	1.6	1.4	**2.2**	1.1
Contig02889	Photosystem II biogenesis protein psp29	1.4	**2.7**	**2.6**	1.3	1.9	1.8	-1.3	1.7	1.3
Contig03429	Photosystem II D2 protein	1.3	1.8	1.7	1.8	**2.4**	**2.1**	1	**2.2**	1.7
Contig14092	Photosystem II protein	1.6	**2.7**	**2**	-1.2	**2.1**	1.8	-1.2	1.5	1.6
Contig13305	Photosystem II reaction centre protein y	-1.3	**3**	**3.5**	1.1	**2.7**	**2.5**	1.4	**2.9**	1.3
Contig01925	Photosystem II reaction centre protein D1/psba	-1.3	**2.5**	**3.8**	1.1	**2.4**	**2.5**	-1.1	**2.7**	1.6
Contig02680	Photosystem II stability assembly factor hcf136	1.4	**3.2**	**2.8**	-1.1	**3.1**	**2.7**	-1.3	1.7	**2.2**
Contig03384	Thylakoid lumen ppiase 40 kDa	1.8	**2.6**	1.6	1.1	**3.6**	**2.7**	-1.6	1.3	**2.3**
Contig15193	Thylakoid lumenal 15 kDa protein	1.3	**2.8**	**2**	-1.1	1.9	**2.4**	1.1	1.8	**2**
Porphyrin and chlorophyll metabolism										
Contig18633	Coproporphyrinoegen III oxidase	1.6	**2.1**	1.6	1.1	**2**	1.2	1	1.2	1.1
Contig06100	Geranylgeranyl diphosphate reductase	**3.9**	**4.2**	**2.9**	1.1	**2.1**	1.2	**-2.5**	-1.6	-1.7
Contig27613	Magnesium-chelatase subunit h	1.8	**3.5**	**2.2**	1.6	**2**	1.3	-1.7	1.6	-1.3
Contig16043	Pheophorbide a oxygenase	1.7	**2**	1.6	1.1	**2.2**	1.4	-1.1	-1.1	-1

Table IV. Differential regulated genes encoding for vitamin B_6 metabolism, and ROS scavenging enzymes. All displayed genes were differentially expressed with $p < 0.01$ and were considered to be significant differently expressed with a fold change > 2 (numbers in bold).

Contig name	Putative gene product	Fold change								
		2°C HP	2°C HPAB	2°C LPAB	7°C HP	7°C HPAB	7°C LPAB	12°C HP	12°C HPAB	12°C LPAB
Vitamin B_6 metabolism										
Contig03286	Pyridoxal 5 -phosphate synthase	1	**4.7**	**4.4**	1.2	**2.9**	**2.2**	1.4	**2.5**	**2.2**
Contig09890	Pyridoxal biosynthesis protein pdx1	**2.1**	**5.3**	1.7	**2.2**	**4.9**	1.4	1.8	**2.6**	1.1
Contig20055	Pyridoxal biosynthesis protein pdx1.3	1.6	**4.4**	**2.1**	**2.3**	**3.2**	1.2	1.6	**3**	1.2
Contig05224	Pyridoxal biosynthesis protein pdx2	1.6	**3.4**	**2.1**	1.8	**2.6**	1.5	1.7	**2**	1.4
ROS scavenging enzymes										
Contig02732	Ascorbate peroxidase	-1.4	-1.3	-1.5	1.8	1.1	-1.2	**2.2**	**2.2**	-1.4
Contig03562	Glutathione reductase	1.5	**2.8**	**2.7**	-1.2	**3**	1.9	-1.1	1.6	1.5
Contig04061	Superoxide dismutase [Fe]	**2.2**	1.8	1.2	1.4	**2**	-1.2	1.7	**2.2**	-1.2
Contig12014	Superoxide dismutase [Mn]	-1.3	1	-1.2	1	1.1	1.1	**2**	**2.1**	1.1
Contig10138	Thioredoxin h	-1.5	1.2	1.1	1.8	1.4	-1.1	**2.2**	**2.7**	-1.1
Contig01732	Thioredoxin reductase	**2.1**	1.9	1.5	1.5	**2.4**	1.4	-1.2	1.5	1.2
Contig14359	Thioredoxin chloroplastic	1.3	**2.3**	**2**	-1.1	1.6	1.4	-1	1.2	1.1

Table V. Experimental light and temperature conditions used for exposure experiments

	Irradiance			**Temperature [C°]**
	PAR [µmol m^{-2} s^{-1}]	**UV-A [W m^{-2}]**	**UV-B [W m^{-2}]**	
Desk 1	23.8 ± 3.08	9.83 ± 1.17	0.42 ± 0.03	2, 7, 12
Desk 2	107.77 ± 4.96	9.68 ± 1.2	0.43 ± 0.03	2, 7, 12

FIGURES

Figure 1. Maximum quantum yield of photosystem II (Fv/Fm) of *Saccharina latissima* after exposure at three temperatures (2,7,12°C) and 4 radiation conditions [low photosynthetically active radiation (PAR) treatments = LP; low PAR+UV =LPAB; high PAR = HP, high PAR+UV = HPAB], measured over 14 days of exposure

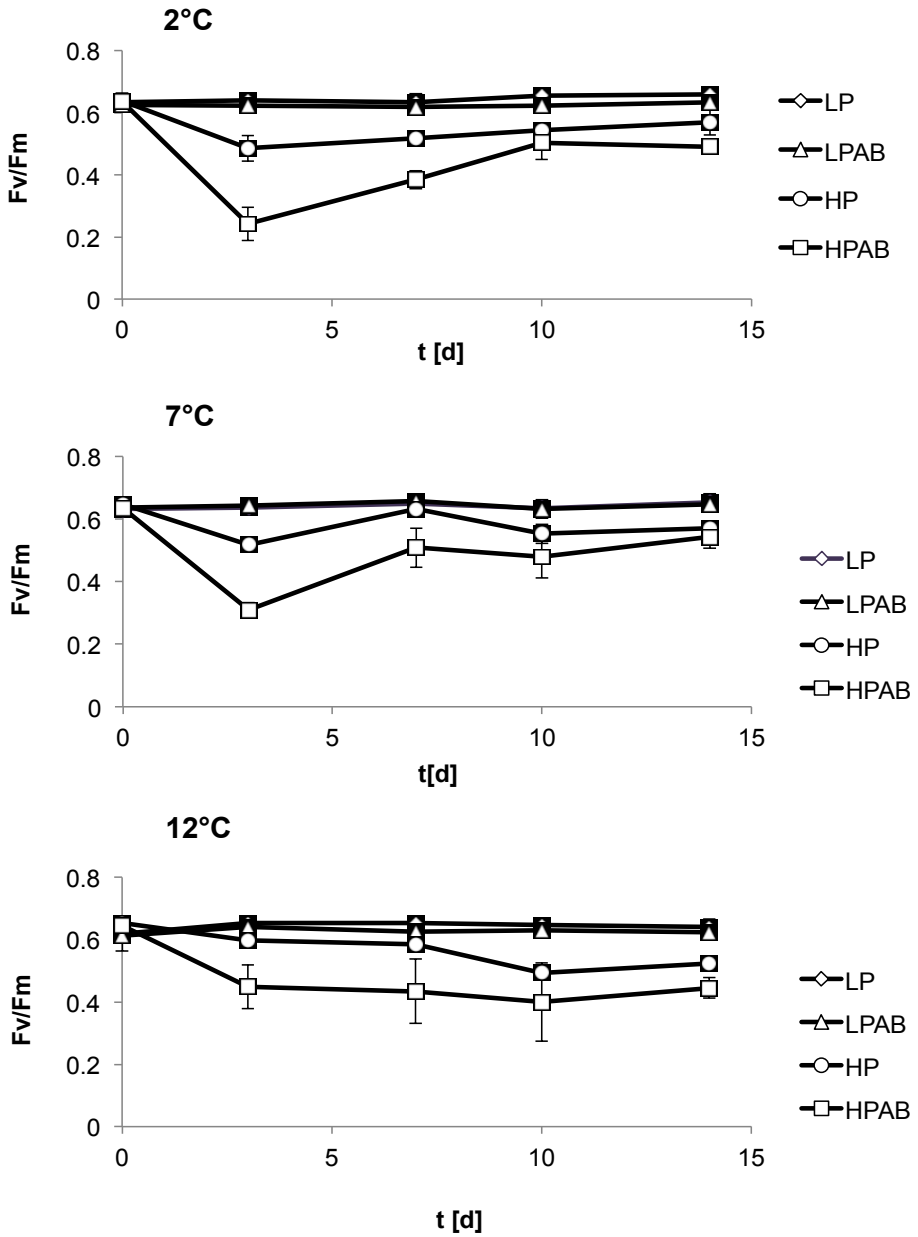
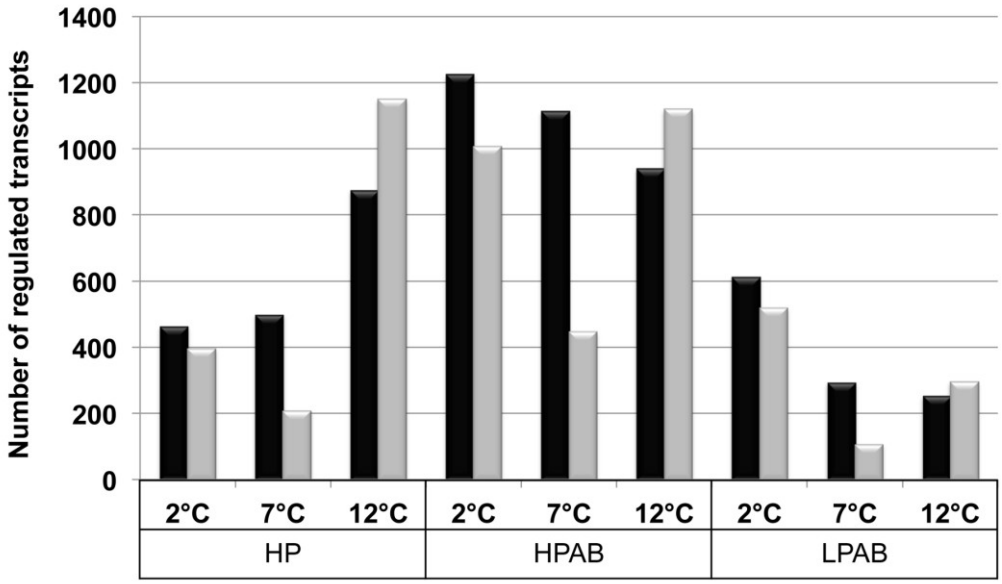


Figure 2. Total number of differentially up- (black bars) and down-regulated (grey bars) genes after exposure to 3 temperatures (2, 7, 12°C) and 3 radiation conditions: low photosynthetically active radiation + UV (LPAB), high photosynthetically active radiation (HP), and high photosynthetically active radiation + UV (HPAB)



Publication IV

(in preparation)

Comparative gene expression in field and cultivated sporophytes of *Saccharina latissima* (Phaeophyceae) exposed to different radiation and temperature conditions

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ABSTRACT

Kelps, brown algae of the order *Laminariales*, dominate rocky shores of cold-temperate regions and constitute important components of cold-temperate coastal ecosystem. Important factors influencing their distribution are light including UV radiation and temperature therefore future global environmental changes could potentially have a significant impact on their zonation, distribution patterns, and primary productivity. An important question is, to what extent results obtained in the laboratory can be used to predict environmental effects in the field. So far only few comparative studies of acclimation in laboratory and field grown macroalgae are published. To explore possible interactive effects of UV radiation, temperature and growth conditions, cultured and field sporophytes of *Saccharina latissima* were exposed for 24h to UV radiation at three different temperatures (2,7 & 12°C). Gene expression profiles under UVR at different temperatures were assessed through microarray hybridizations, afterwards comparisons of gene expression profiles in field and culture sporophytes were conducted. Principal effects of UVR, targeting mostly photosynthesis and DNA, were similar in culture and field sporophytes, demonstrating laboratory experiments being well suited for investigating basic molecular mechanisms of acclimation to abiotic stresses. Field sporophytes exhibited a higher susceptibility to UVR and a higher oxidative stress level at 12°C, whereas cultured sporophytes in contrast were more prone to UVR at 2°C. These findings are mostly due to the different growth temperatures of field and cultured sporophytes. The results indicate that cold acclimation of *S. latissima* from the field caused metabolic alterations to increase stress performance at low temperatures, which concurrently led to higher susceptibility at 12°C.

INTRODUCTION

Global environmental changes, e.g. global warming and increased UV radiation due to depletion of the stratospheric ozone layer, represent major threats especially to polar marine ecosystems (Bischof et al., 2006; Harley et al., 2006; Bartsch et al., 2012). According to the future high scenario of the Fourth Assessment Report of the United Nations Intergovernmental Panel on Climate Change a global average surface warming in the range of 2.4–6.4°C is predicted by 2100. The warming is expected to be strongest at high northern latitudes and least in the Antarctic region (IPCC, 2007). Since 1950 mean annual sea surface temperatures in the North Atlantic have risen by more than 1.5°C, which is more than twice of the global average (Merzouk and Johnson, 2011). Within the last years, depletion of the stratospheric ozone layer has caused enhanced UV-radition at the earth`s surface, these increases are especially pronounced at high latitudes (Kerr and McElroy, 1993; Madronich et al., 1998). Only recently, an unprecedented ozone loss has been demonstrated over the Arctic (Manney et al., 2011). At the worst case future scenario an ozone depletion over the Arctic up to 20 % until 2020 is forecasted (WMO, 2006).

There is consensus that coastal marine ecosystems are endangered by global climate changes (IPCC, 2001; Helmuth et al., 2006). Benthic macroalgae constitute important components of marine coastal ecosystems in providing food for herbivores and detritivores, as well as a habitat for many sessile and motile organisms (Lüning, 1990; Lippert et al., 2001; Carlsen et al., 2007). In addition macroalgae account for up to 10% of the global oceanic primary production (Smith, 1981; Charpy-Roubaud, 1990; Lüning, 1990; Carlsen et al., 2007). Primary abiotic factors for determining vertical zonation and geographical distribution of macroalgae are temperature, and light, including UV radiation (Hanelt, 1998; Wiencke et al., 2006; Bartsch et al., 2008). It is thus likely that the increase of UV radiation and sea surface temperatures will influence distribution patterns of benthic macroalgae. Latitudinal shifts in species distribution of macroalgae as ecological responses to climate changes have been already observed (Simkanin et al., 2005; Lima et al., 2007; Hawkins et al., 2009).

UV radiation affects negatively photosynthesis, leading to degradation of light harvesting complex proteins, D1/D2 heterodimer of photosystem II, and influence secondary photosynthetic reactions (Bischof et al., 1998; Bischof et al., 2000; Wiencke et al., 2000; Karsten et al., 2001; Bischof et al., 2002; Franklin et al., 2003; Davison et al., 2007).

Another key target of UV radiation is DNA, where it can cause e.g. double-strand breaks of

DNA, crosslinks between DNA-DNA, and formation of cyclobutane-pyrimidine dimers (CPDs) (Hall et al., 1992; Britt, 1999; Sinha and Häder, 2002). Studies in macroalgae demonstrated that exposure to UV radiation leads to formation of CPDs, which are removed by photoreactivation and nucleotide excision repair (Pakker et al., 2000; Van De Poll et al., 2002). Furthermore it was shown, that UV susceptibility of macroalgae seems to be life-stage dependent, and is closely related to their vertical distribution (Karsten et al., 2001; Roleda et al., 2006; Roleda et al., 2007; Wiencke et al., 2007). While UV damaging effects on seaweed physiology are well studied in single factor experiments, only few projects focused on interactions between UVR and other abiotic factors. Simultaneous exposure to several stress factors can elevate their damaging effects, and might lead to an increase of susceptibility to additional stresses (Alexieva et al., 2003; Wernberg et al., 2010). Previous studies demonstrated interactive effects of temperature and UV radiation on germination in macroalgae, here germination rates increased with rising temperatures as long as the temperatures did not approach upper thermal maxima (Hoffman et al., 2003; Müller et al., 2008). For some macroalgae species it was shown, that photosynthetic efficiency under UV radiation was higher at moderate temperatures compared to low temperatures (Rautenberger and Bischof, 2006; Fredersdorf et al., 2009). These results underline the importance of multifactorial experiments, especially for investigating climate change effects. Another important question is, to what extent results obtained in the laboratory can be used to predict environmental effects in the field. Until now only few comparative studies of acclimation in laboratory and field grown macroalgae are published (Bischof et al., 1999; Sagert and Schubert, 2000).

The purpose of the present study is to explore possible interactive effects of UV radiation and temperature on gene expression in *Saccharina latissima*, and to determine how the growth conditions of the algae in the field and in the laboratory influence gene expression profiles under UVR and temperature stress. The object of our study, the perennial kelp *Saccharina latissima*, is a common species in kelp beds of Arctic and cold-temperate coastal waters (Bolton et al., 1983; Gerard, 1988; Borum et al., 2002). For comparing possible interaction in respect to origin of sporophytes, all experiments were conducted with laboratory grown and field grown *S. latissima*, which was collected in Spitsbergen (Norway).

MATERIAL & METHODS

Algal material

Field material

Sporophytes of *Saccharina latissima* with a size of 5-7cm were collected in May 2009 directly after the sea-ice breakup by scuba diving from 8m water depths in Kongsfjorden (79°N; 11°E; Svalbard, Norway). The water temperature at that time was 1°C. Algae were transported to the laboratory in black plastic containers, were cleaned from epiphytes, and kept for 48h in running seaweater at 3-5°C under low light (10 µmol photons m^{-2} s^{-1}) prior to the exposure experiments.

Culture material

Unialgal cultures of gametophytes of *Saccharina latissima*, originally established from spores of fertile sporophytes collected by SCUBA diving in Kongsfjorden (79°N; 11°E; Svalbard, Norway, AWI culture numbers: 3123, 3124), were used to raise young sporophytes. Filaments of male and female gametophytes were fragmented together, transferred to Petri dishes filled with Provasoli enriched seawater (PES) (Starr and Zeikus, 1993) and cultured at 10 +/- 1°C and 30 µmol photons m^{-2} s^{-1} white light at 18h light: 6h dark period. Developing sporophytes were transferred after 2 weeks to aerated 5l culture bottles and grown in PES until they reached a size of 5-7cm.

Light & temperature treatments

Irradiation experiments were conducted in environmentally controlled rooms at 2, 7 and 12°C ± 1°C. Young sporophytes from different origin were exposed for 24h to low photosynthetically active radiation (23.6 ± 3 µmol photons m^2 s^{-1}) in combination with UV radiation (UV-A: 10.56 ± 1.04 W m^{-2}; UV-B: 0.45 ± 0.02 W m^{-2}). Every experiment was conducted with 5 replicates. For distinguishing the effects of different wavelength ranges, the experimental units were covered with cut-off filter foils transparent to wavelengths of: (1) 400- 700 nm (URUV Ultraphan UV farblos, Difrega, Germany) for photosynthetically active radiation (PAR) treatments, and (2) 295-700nm (URT 140 Ultraphan UV farblos, Difrega) for PAR + UVA + UVB treatments. PAR was provided by 3/5 Osram daylight fluorescent tubes (Biolux, 36W; Osram, Germany) and measured by using a LI- 250 light meter (LI-COR, Lincoln; USA). UV radiation was generated by 3 fluorescent tubes (UV A-340, 40W; Q-Panel, USA) and determined with a Solar Light PMA-2100 (Solar Light; PA, USA).

Fluorescence measurements

As indicator of the physiological status of the algae, maximum quantum yield of PS II (Fv/Fm) was measured in the beginning and at the end of the experiment with an Imaging PAM (Pulse Amplitude Fluorometer, Walz, Effeltrich, Germany). Prior to the measurements sporophytes were dark-adapted for 5 min.

Subsequent to the fluorescence measurements sporophytes were frozen in liquid nitrogen and stored at -80°C until RNA extraction. Results of the photosynthetic measurements were analysed by analysis of variance (ANOVA) with repeated measurements ($p < 0.01$). Significant differences as well as interaction of means were compared with the post hoc Tukey test (HSD, $p < 0.01$). Statistical analysis was performed using SPSS software version 19 (IBM, USA).

RNA-extraction and -labelling

Total RNA extraction from differently treated sporophytes was performed as described by Heinrich et al. (2012), consisting of a CTAB extraction, followed by the use of a Quiagen RNA isolation kit. Total RNA was labelled with the Agilent two-colour Low Input Quick Amp Labeling kit (Agilent Technologies, Waldbronn, Germany), control treatment RNA was labelled by fluorescent complementary RNA (cRNA) synthesis with cyanine-3-CTP, whereas RNA from stress treatments was labelled with cyanine-5-CTP. Agilent RNA Spike-In Mix (Agilent) was added to 200 ng of total RNA prior to the labelling. On account of the extensive length of 3`untranslated regions (UTRs) occurring in brown algae, cDNA synthesis was performed using a blend of T7 nonamer primer and T7 promoter primer in equal molarity. cRNA synthesis and purification of labelled RNA was conducted following the two-colour Low Input Quick Amp Labeling kit protocol (Agilent). cRNA yield, as well as dye incorporation rates of cyanine-3 and cyanine-5, was measured with a NanoDrop ND-100 spectrometer (PeqLab, Erlangen, Germany).

Microarray design and hybridization

Agilent's eArray online application tool was used to design 6x80 k microarrays slides, containing 60mer oligonucleotides probes created from a *Saccharina latissima* EST library, which was established from RNA sampled under several light and temperature conditions (Heinrich et al., 2012). Overall 25,262 transcripts were represented on the microarray by 1, 2 or 3 individual probes. Hybridizations for all experimental conditions were performed in 4 replicates.

Total RNA samples derived from the treatments were hybridized against a pooled control consisting of RNA from the low PAR 2/7/12°C treatments. The microarray hybridization procedure was carried out with 300ng of cyanine-3 and cyanine-5 labelled cRNA for 17h at 65°C. Subsequently microarray disassembly and wash procedure followed as described by the manufacturer's instructions (Agilent). Microarrays were scanned with the Agilent G2565AA scanner. Raw data were processed with the Agilent Feature Extraction Software version 9.1.3.1 (FE); array quality was monitored using the Agilent QC Tool (v1.0) with the metric set GE2_QCMT_Feb07. The microarray design, raw data and normalized data as well as the detailed experimental design are MIAME compliant and deposited in a MIAME compliant database (ArrayExpress at the EBI; http://www.ebi.ac.uk/microarray-as/ae/; ID: E-MEXP-xxx).

Statistical analysis of microarray data

Testing for differential expressed genes was conducted using the GeneSpring GX software platform version 11 (Agilent) with the implemented statistical tests. At this an ANOVA, followed by a post hoc test Tukey HSD with the Benjamini Hochberg FDR correction was performed. Genes were considered to be differential expressed when test statistics reply *p*-values were less than 0.01 and calculated fold changes between the control and the treatment was at least 2.

Enrichment of GO annotations within the regulated genes of the various exposure treatments was assessed by gene set enrichment analysis, which was done using Blast2GO (Conesa et al., 2005). Blast2GO applies the Fisher's exact test including corrections for multiple testing using FDR (false discovery rate), FWER (family-wise error rate) and single test *p*-value. Over-represented KEGG pathways were identified by KOBAS (http://kobas.cbi.pku.edu.cn/home.do) using a hypergeometric test ($p < 0.01$).

RESULTS

Photosynthetic measurements

Fv/Fm was affected by temperature, light, as well as by interaction of light and temperature (table S1). No significant difference in maximum quantum yield of PS II (Fv/Fm) between cultured and field material, neither at the beginning nor after the exposure experiments. Initial mean maximum quantum yield (Fv/Fm) of cultured sporophytes and field sporophytes was 0.631 ± 0.03 respectively 0.637 ± 0.03. Maximum quantum yield of PS II remained unchanged after exposure to low photosynthetically active radiation (PAR) under the three tested temperatures (2,7,12°C; Fig. 1). Exposure to low PAR + UV radiation significantly reduced maximum quantum yield, at this photoinhibition was significantly stronger ($p < 0.01$) at 2°C (Fv/Fm culture = 0.129 ± 0.1; Fv/Fm field = 0.184 ± 0.003) compared to 12°C (Fv/Fm culture = 0.418 ± 0.104; Fv/Fm field = 0.39 ± 0.177). At 2 and 7 °C Fv/Fm in LPAB irradiated material was slightly but insignificantly higher in the field compared to the cultured material. This trend was, however, not present at 12 °C.

Microarray analysis

Gene expression profiles under UV stress at different temperatures were obtained by using oligonucleotide microarrays, which covered 25,262 transcripts. Comparing the overall number of significant regulated transcripts of cultured and field sporophytes in response to the exposure conditions, we detected large difference in the number of regulated transcripts (Fig. 2). Exposure of cultured *S. latissima* to UV radiation (UVR) led to a changed expression of 8,166 transcripts (32%) in at least one stress treatment compared to the control treatment (2,7,12°C & low PAR), the strongest effect on gene expression was observed after UVR exposure at 7°C treatment, when 3,770 genes (15%) were affected. Furthermore we observed strong interactive effects of temperature and UVR in cultured sporophytes: at 12°C the amount of regulated genes was only half as much compared to the 2°C and 7°C treatment.

In field material of *S. latissima* 1,218 transcripts (5%) showed different expression patterns under at least one stress treatment compared to the control treatment (2,7,12°C & low PAR), at this highest number of regulated transcripts (1014≈ 4%) was detected after the UV treatment at 2°C.

Gene Ontology term enrichments

To investigate the composition in terms of gene function of all significantly up- and down-regulated transcripts of the different stress conditions, Gene Ontology (GO) term enrichments were conducted. Among the regulated transcripts of the different stress conditions in cultured sporophytes of *S. latissima* we detected a total of 164 over-represented GO terms, at this highest number of enriched GO terms was found within the up-regulated genes after exposure to UV radiation at 2°C (table 1). In field material of *S. latissima* a total of 67 enriched GO terms was identified among the regulated transcripts of the different treatments, with the highest number of over-represented GO terms occurring among the regulated transcripts after UV exposure at 12°C. Despite the large difference in the number of detected enriched GO terms among the regulated genes in cultured and field material of *S. latissima*, we found similarity in terms of function. The majority of over-represented GO terms among the up-regulated transcripts of all UVR treatments were either correlated to photosynthetic components (e.g. chloroplast GO:0009507, thylakoid GO:0009579), DNA repair (e.g. recombinational repair GO:0000725, double-strand break repair via homologous recombination GO:0000724), or DNA replication (e.g. DNA replication factor A complex GO:0005662, nuclear replisome GO:0043601). Cultured sporophytes of *S. latissima* featured highest number of enriched GO terms related to nucleotides in response to UV at 2°C, where we found 16 enriched terms; followed by the conditions UV at 7°C and 12°C, with 11, respectively 4, over-represented GO terms. In field material of *S. latissima* 7 over-represented GO terms correlated to nucleotides occurred after UV exposure at 7°C, whereas the treatments UVR at 2°C and 12°C featured 4, and accordingly 6, enriched GO terms. For a detailed list see Table S2.

We identified several more enriched GO terms among the regulated transcripts in cultured sporophytes. Enhanced induction of genes associated with vitamin metabolic processes (e.g. vitamin B_6 biosynthetic process GO:0042819), and cellular anion homeostasis, like monovalent inorganic anion homeostasis (GO:0055083), was observed after exposure to UVR at 2°C and 7°C. These treatments furthermore caused repression of transcripts correlated to carbohydrate metabolic processes, like glucan metabolic process (GO:0044042), and polysaccharide metabolic process (GO:0005976). Further features of the UVR 7°C treatment were the enhanced repression of transcripts correlated to endocytosis (GO:0006897), endosome transport (GO:0016197) and vesicle-mediated transport (GO:0016192) as well as repression of genes associated with clathrin-dependent endocytose, e.g. clathrin coat

(GO:0030118) clathrin-coated vesicle (GO:0030136), and clathrin coated vesicle membrane (GO:0030665).

KEGG pathway analyses

We identified 7 significantly enriched metabolic pathways by KOBAS analysis within the UVR treatments at different temperatures (table 2). Exposure of cultured material to UV radiation at 2°C led on the one hand to up-regulation of transcripts involved in ABC transport and glutathione metabolism, on the other hand to repression of alanine, aspartate and glutamate metabolism, galactose metabolism, glycolysis/ gluconeogenesis, as well as of the pentose phosphate pathway. Among the up-regulated genes in field sporophytes under UVR at 2°C and 7°C increased regulation of carotenoid biosynthesis was observed.

Manual analysis of UVR-induced transcripts

As GO term enrichment analysis indicated enhanced induction of transcripts correlated with photosynthetic components, DNA replication, DNA repair, and vitamin B_6 biosynthesis in response to UVR, we manually analysed transcriptional changes of genes encoding for these categories (table 3). To gain insights into the oxidative stress level during UVR stress in *S. latissima*, we inspected the expression of reactive oxygen species (ROS) scavenging enzymes (table 3). A full list of the regulated genes with annotations can be retrieved from the supplemental material (table S3). Cultured sporophytes featured higher number of regulated transcripts after UVR exposure as field sporophytes, especially within the categories photosynthetic components, DNA repair, and ROS scavenging enzymes. To investigate whether the observed interactive effect of algae origin and UVR not only influences the amount of regulated genes, but also the level of expression fold changes of genes, we focused on transcripts, which were simultaneous expressed in cultured and field material.

We detected 11 transcripts encoding for photosynthetic components, which were significantly regulated after exposure to UV radiation. Regulation of all these transcripts was observed in cultured material after exposure to UV at 7°C, in field material after exposure to UV at 2°C. Transcripts of the cytochrome b6 complex (contig02465) were induced in response to all experiments with transcriptional changes between 2.2 – 3.2 fold, with the highest transcript abundance in field sporophytes exposed to UVR at 12°C. We observed regulation of three transcripts encoding for light harvesting proteins. Fucoxanthin-chlorophyll a-c binding protein (contig13579) was significantly induced in all treatments except after the 12°C UVR treatment in cultures, with the highest up-regulation of 253.9 fold occurring in field material

exposed to UVR at 2°C. Light harvesting complex protein (contig24218) and light harvesting complex I 21 kDa (contig08085) were repressed in cultured material at 2°C and 7°C UVR, in field material after exposure to UVR at 2°C; light harvesting complex I 21 kDa was additionally down-regulated in field material in response to UVR at 12°C. Photosystem I reaction center subunit II featured enhanced transcript abundance in field sporophytes after all UVR conditions, in cultured material only in response to UVR at 7°C. We detected 4 regulated genes correlated to photosystem II: Photosystem II D2 protein (contig03429) was induced in all treatments, photosystem II biogenesis protein psp29 (contig02889), and photosystem II stability assembly factor hcf136, (contig02680) were up-regulated after all treatments except the culture 12°C UVR treatment, respectively the field 12°C UVR treatment. Photosystem II protein (contig14092) showed higher transcript abundance in cultured material exposed to UVR at 7°C, as well as in field material exposed to UVR at 2°C. Additionally we observed up-regulation of the chloroplastic thylakoid lumenal 15 kDa protein (contig03555) in all experiments conducted with field sporophytes, and in response in cultured material after UVR at 7°C. Furthermore induction of thylakoid lumenal protein was detected in field sporophytes exposed to UVR at 2°C and 7°C, and cultured sporophytes exposed to UVR at 7°C.

We identified 4 regulated transcripts associated with DNA repair; rad51 DNA recombination repair protein (contig07877) and x-ray repair cross-complementing protein 6 (contig06170) were induced in all treatments apart from cultured material subjected to UVR at 12°C, at this x-ray repair cross-complementing protein 6 showed higher fold changes in field sporophytes than in cultured sporophytes. A deoxyribodipyrimidine photolyase family protein (contig21643) was up-regulated in all treatments except in cultured material exposed to UVR at 12°C, highest fold changes of these transcripts were detected in 2°C UVR treatments of both origins. Induction of DNA repair protein uvh3 (contig04132) was observed in all treatments except in field material of the UVR at 7°C condition, highest transcript abundance in cultured material occurred after the 2°C UVR treatment, whereas field material featured highest transcript abundance of this gene under UVR at 12°C.

We detected significant up-regulation of several transcripts involved in DNA replication in response to the stress treatments, but no significant down-regulation within this category. Replication factor-a protein (contig20298), replication protein-a 69 kDa DNA-binding subunit (contig07889), and replication protein-a 70 kDa DNA-binding (contig13754) were induced in response to all experimental conditions, the highest transcript abundance was observed in cultured sporophytes after exposure to UVR at 2°C, field sporophytes on the contrary showed

highest transcript abundance in response to UVR at 12°C. Furthermore fold changes of these transcripts were higher in cultured material exposed 2°C UVR as in field material exposed 2°C UVR. We detected up-regulation of DNA gyrase subunit b (contig20059) and DNA polymerase I (contig05303) after all UVR treatments apart from cultured material subjected to UVR at 12°C, at this highest transcript abundance in cultured material was found after the 7°C UVR treatment, whereas field material showed highest transcript abundance of this gene under UVR at 2°C. Ribonucleoside-diphosphate reductase large chain (contig23469) was induced in field sporophytes after all UVR treatments, in cultured sporophytes only after UVR exposure at 2°C. Ribonucleoside-diphosphate reductase small chain (contig02084) was up-regulated after all UVR experiments conducted with field sporophytes, cultured sporophytes featured up-regulation in response to UVR 2°C. We detected stronger induction of both genes in response to 2°C UVR in cultured material compared to field material.

We identified two up-regulated transcripts correlated to vitamin B_6 metabolism, pyridoxal biosynthesis protein pdx2 (contig25393), and pyridoxamine 5-phosphate oxidase (contig03286), which were both up-regulated in response to all UVR treatments. At this juncture transcript abundance at 2°C UVR was higher in cultured material as in field material, in contrary to the 12°C UVR treatment, where induction was stronger in field sporophytes as in cultured sporophytes.

Five genes encoding for antioxidative enzymes were simultaneously regulated in cultured as well as field sporophytes. Chloroplastic alternative oxidase (contig10729) was induced in response to all treatments with expression changes between 2.2-fold and 4.9-fold, strongest induction occurred in cultured and field sporophytes after exposure to UVR at 2°C. Furthermore, UVR at 2°C and 7°C caused stronger induction in cultured material as in field material, under UVR at 12°C field material featured stronger up-regulation. Enhanced transcript abundance of dehydroascorbate reductase (contig06154) was observed in cultured material after exposure to UVR at 2°C and 7°C, and in field material in response to UVR at 2°C and 12°C.

Algae from both origins featured induction of l-ascorbate peroxidase (contig00051) after the 2°C/7°C UVR treatment. Additionally we detected enhanced transcript abundance of thioredoxin reductase after all treatments except in cultured sporophytes of the UVR at 12°C condition, with stronger up-regulation after the 2°C/7°C UVR treatment occurring in cultured material as in field material.

DISCUSSION

Similarities and differences of transcriptomic stress response to UVR in cultured and field sporophytes of *S. latissima*

This project represents the first comparative gene expression study in cultured and field material of seaweeds in response to UV radiation (UVR) stress at different temperatures. Our aim was to investigate how the exposure conditions in the field and in culture influence gene expression profiles under UVR and temperature stress. We observed large differences in the overall number of regulated genes. Cultured material responded to the treatments with significant expression changes of 8,166 (32%) genes, while in field material 3,770 (15%) transcripts were regulated. We observed strong interactive effects of temperature and UVR on gene expression in cultured sporophytes, at 12°C the amount of regulated genes was only half as much compared to the 2°C and 7°C treatment. Field material in contrast did not exhibit such strong interactive effects of UVR and temperature in gene expression. The higher number of regulated genes, enriched GO terms, and over-represented KEGG metabolic pathways found in cultured material subjected to UVR indicates that cultured sporophytes must make stronger efforts of acclimating to UVR than field sporophytes. One reason for this might be the different age of the thalli, with an age of 8 weeks for cultured sporophytes versus 4 month for field sporophytes. Studies on *S. latissima* revealed differences in UVR sensitivity with respect to the age of the thalli, which is partly due to age-dependent morpho-functional features (Dring et al., 1996; Bischof et al., 2002). Field plants were taken directly after the ice break-up, and should be therefore sensitive to UVR. However our results suggest, that field sporophytes are less sensitive to UVR than cultured sporophytes. For plants it was shown, that exposure to a single stress agent can lead to increased resistance to subsequent unfavourable impacts (Alexieva et al., 2003). This might be also the case for field sporophytes of *S. latissima*, exposure to cold temperatures might led to an increased resistance to UV-B radition. In genes simultaneous regulated in cultured and field sporophytes, differences in the level of expression fold change in response to similar stress conditions were observed, which might be due to the different growth temperatures of between -2°C and 1°C for field sporophytes versus 10°C for cultivated sporophytes. Hence the field algae had to acclimate to higher temperatures (7 and 12 °C), whereas the cultured algae had to acclimate to lower temperatures (2 and 7 °C). Our results demonstrate the influence of growth conditions on the acclimation to stress on the transcriptional level. Physiological studies on *S. latissima* showed, that increasing growth temperature led to higher temperature tolerance of photosynthesis, partly

due to changes in RubisCO kinetics and thermal stability of light harvesting electron transport systems (Davison, 1987; Davison and Davison, 1987). Furthermore it has been suggested, that in *S. latissima* complex metabolic regulations are responsible for optimizing photosynthesis over a wide range of temperature and light conditions (Machalek et al., 1996). However, in both cultivated and field sporophytes results of photosynthetic measurements and microarray analysis indicate a temperature optimum for photosynthesis of 12°C. This result agrees with previous studies, which showed that the optimum growth range for *S. latissima* is between 10°C and 15°C (Fortes and Lüning, 1980; Bolton and Lüning, 1982; Lüning, 1984).

Similarities of transcriptomic response to UVR in cultured and field sporophytes include enhanced regulation of photosynthetic components, DNA repair and DNA replication. Several studies on UV stress in macroalgae demonstrated, that UVR influences photosynthesis negatively and causes DNA damage (Karsten et al., 2011). Our transcriptomic data supports these findings, indicating photosynthesis and DNA being prime targets of UVR in macroalgae.

We observed induction of vitamin B_6 biosynthesis after all UVR treatments. Vitamin B_6 is a water-soluble vitamin, which possesses high antioxidant activity, and is suggested to be involved in protection from oxidative stress (Ehrenshaft et al., 1999; Mooney and Hellmann, 2010). In *Arabidopsis* vitamin B_6 was demonstrated to be crucial for acclimation to osmotic and oxidative stress (Shi et al., 2002; Chen and Xiong, 2005). We suggest that vitamin B_6 might be involved acclimation to UVR in *S. latissima*, and might be participating in protection from oxidative stress.

Maximum quantum yield of PS II and transcription of photosynthetic components under UVR stress

Exposure to UVR caused in all experiments presented here significant decreases in photosynthetic efficiency. We observed interactive effects of UVR and temperature, with the highest degree of photoinhibition occurring in sporophytes of both origins after UVR exposure at 2°C, despite the different growth conditions of cultivated and field sporophytes. Our results of stronger photoinhibition under UVR at low temperatures are consistent with previous studies on the interaction of UVR and temperature in macroalgae. Germination rates of *Alaria marginata* and *Fucus gardneri* increased under UVR stress with rising temperatures (Hoffman et al., 2003). Müller et al. (2008) investigated the interactive effects of UVR and temperature on germination of spores in different kelp species. In their study, *S. latissima* exhibited higher germination rates under UVR at 12°C compared to 2°C. Studies on interactive effects of UVR and temperature on photosynthetic efficiency in macroalgae showed similar results, the degree of photoinhibition in two investigated two *Ulva* species

was smaller at 10°C than at 0°C (Rautenberger and Bischof, 2006). Sporophytes of the kelp species *Alaria esculenta* featured higher maximum quantum yield of PS II under similar UV-B radiation conditions at 13°C and 17 °C compared to 4°C and 9°C (Fredersdorf et al., 2009). We observed no interactive effects of origin and stress treatments on maximum quantum yield in *S. latissima*, although sporophytes of different origin experienced different growth temperatures (-2°C and 1°C for field sporophytes versus 10°C for cultivated sporophytes). Comparative studies of acclimation to stress in laboratory and field grown macroalgae are rare. Sagert and Schubert (2000) investigated acclimation to light intensity of field and laboratory grown *Palmaria palmata*, they observed differences in pigmentation trends between field and laboratory material, but no differences in light saturation points (E_k) of photosynthesis. Studies on maximum quantum yield and maximum electron transport rates under UV stress in *Alaria esculenta* from field and culture revealed, that the course of acclimation to UVR is similar in plants from field and culture (Bischof et al., 1999).

Even though origin of the sporophytes did not significantly influence the maximum quantum yield of photosystem (PS) II, it led to differences in the overall number of regulated genes encoding photosynthetic components The absolute number was higher within the treatments conducted with cultured material; furthermore we observed different expression fold changes of genes being simultaneous regulated in cultivated and field material. Thylakoid proteins were induced in a higher number of treatments in field sporophytes compared to cultured sporophytes. Transcripts encoding for light harvesting complex proteins showed diverse regulation patterns in response to UVR, fucoxanthin-chlorophyll a-c binding protein was stronger induced in field material, while light harvesting complex protein and light-harvesting complex I 21 kDa showed stronger repression in sporophytes from culture. We found 4 genes associated with photosystem II and one gene correlated to photosystem I. Photosystem I reaction center subunit II was induced in all field sporophyte experiments, but only after one treatment in cultured material, indicating a higher protein turnover rate of PS I reaction centre under UVR in field sporophytes compared to cultivated sporophytes. Transcripts associated to photosystem II, e.g. photosystem II D2 protein, were up-regulated after most of the UVR treatments, usually with higher induction in field material. The higher number of regulated transcripts, as well as higher number of treatments featuring regulation of these genes indicates, that photosystem II is more sensitive to UVR than photosystem I. This agrees with former studies, which demonstrated that PS II is more prone to UVR than PS I (Franklin et al., 2003; Vass et al., 2005). Furthermore it was shown in several studies on macroalgae that UVR leads to degradation of several photosynthetic components, and recovery mechanisms

include degradation and biosynthesis of damaged photosynthetic reaction centre proteins (Franklin et al., 2003; Bischof et al., 2006). For cyanobacteria it was demonstrated that repair of UVR damaged PS II reaction center by increasing the transcript pool (Campbell et al., 1998; Huang et al., 2002). We therefore suggest that the induction of transcripts encoding for PS II and PS I might be reflecting enhanced repair rates of photosynthetic proteins due to an increased turnover rate under UVR stress. The observed differences in gene expression of photosynthetic components under UVR in cultured and field sporophytes of *S. latissima* might be, despite of the similar size of the thalli, caused by differences in age-dependent morpho-functional features of field and cultivated sporophytes. This finding is consistent with a previous study, where it was shown that the acclimation to high irradiance increases with increasing age of sporophytes in *Saccharina latissima* (Hanelt et al., 1997).

UVR effects on DNA replication and repair

UVR exposure caused in all treatments to induction of transcripts correlated to DNA replication and DNA repair. At this higher number of regulated transcripts, and higher number of enriched GO terms of this category, were detected in cultured material.

Most genes involved in DNA replication, e.g. DNA gyrase subunit b and DNA polymerase I, were induced after all UVR treatments except in cultivated sporophytes subjected to UVR at 12°C. This agrees with studies on the effect of UV radiation on gene expression in *Synechocystis sp* and *Arabidopsis thaliana,* which revealed up-regulation of genes coding for DNA replication (Huang et al., 2002; Molinier et al., 2005). Transcripts correlated to replication factor protein-a, like replication factor-a protein and replication protein-a 70 kDa DNA-binding, were induced after all UVR treatments, with highest transcript abundance in cultured sporophytes after exposure to UVR at 2°C, in field sporophytes after UVR at 12°C. Replication protein-a is a single-stranded DNA binding protein, which is involved in DNA replication, DNA-repair and recombination (Wold, 1997). It is essential for nucleotide excision repair, where it interacts with DNA repair enzymes at sites of DNA damage, and repair of double-strand breaks by homologous recombination, here it catalyses the homologous pairing and strand-exchange steps of homologous recombination (Buschta-Hedayat et al., 1999; Stauffer and Chazin, 2004). Studies on *Oryza sativa* and *Arabidopsis thaliana* revealed different types of replication protein-a 70 kDa, transcripts encoding for these proteins showed induction in response to high levels of UV, and are therefore suggested to be required for repair of UV-damaged DNA (Ishibashi et al., 2001; Ishibashi et al., 2005). We found four genes encoding for DNA repair enzymes, three of them showed enhanced

transcript abundance after all UVR treatments except in cultured sporophytes after the UVR 12°C condition. Induction profiles of these transcripts were dependent on temperature and origin, but also on interaction of these two factors. A deoxyribodipyrimidine photolyase family protein showed highest induction in both origins after the 2°C UVR treatment, x-ray repair cross-complementing protein 6 showed generally higher fold changes in field sporophytes than in cultivated sporophytes, whereas DNA repair protein uvh3 featured highest transcript abundance in cultured material after the 2°C UVR treatment, in field material after the 12°C UVR condition. These results indicate that the experimental UVR radiation led to severe DNA damage. UVR induces oxidative damage to DNA, double-strand breaks of DNA, crosslinks between DNA-protein and DNA-DNA and enhanced formation of cyclobutane-pyrimidine dimers (CPDs) (Hall et al., 1992; Britt, 1999). CPDs inhibit genome replication and gene expression as a consequence disruption in cell metabolism and division occurs (Buma et al., 1995; Van De Poll et al., 2001). In plants CPDs are predominantly repaired by photoreactivation, a light dependent process during which photolyases bind specifically to CPDs and directly reverse the damage (Britt, 1996). Studies on CPD formation and repair in macroalgae indicate that formation of CPDs in response to UVR is not dependent on temperature. CPDs are removed by photoreactivation and nucleotide excision repair (Pakker et al., 2000; Van De Poll et al., 2002). A study on interactive effects of UVR and temperature on photoreactivation in *Palmaria palmata* demonstrated that light dependent removal of CPDs increases with rising temperatures (Pakker et al., 2000). We suggest the similar case for *S. latissima*, as the highest induction of deoxyribodipyrimidine photolyase family protein occurred under UVR at 2°C, indicating that light dependent photoreactivation is slower at 2°C than at 12°C.

We observed up-regulation of rad51 DNA recombination repair protein, which is involved in homologous recombination, a mechanism for repairing double-strand breaks and single strand gaps in damaged DNA (Sinha and Häder, 2002; Markmann-Mulisch et al., 2007). Furthermore induction of DNA repair protein uvh3 was detected, a putative single-stranded DNA endonuclease involved in nucleotide excision repair of UV- and oxidative damaged DNA (Liu et al., 2001). Our data indicate that acclimation to UVR in *S. latissima* includes sophisticated regulation of three different DNA repair processes, namely photoreactivation, homologous recombination and nucleotide excision repair. A striking feature was that a temperature of 12°C seems to ameliorate the negative effects of UVR on DNA in cultured sporophytes, but not in field sporophytes. This suggests a higher susceptibility of field material to UVR at high temperature compared to cultivated material.

Oxidative Stress

We identified several regulated genes encoding for reactive oxygen species (ROS) scavenging enzymes. UV radiation, and other physiological stress conditions, triggers the synthesis of ROS (Contreras et al., 2009; Kumar et al., 2010). ROS, such as singlet oxygen and hydrogen peroxide, are highly toxic and cause cellular damage by denaturation of, e.g. nucleic acids and proteins therefore they are rapidly detoxified by cellular non-enzymatic and enzymatic scavenging mechanisms (Asada, 1997; Apel and Hirt, 2004; Foyer and Noctor, 2005). Several studies demonstrated the involvement of ROS species in response to biotic and abiotic stresses in macroalgae; increased activity of ROS scavenging enzymes has been shown after exposure to UVR stress, low temperature and desiccation (Collén and Davison, 2001; Aguilera et al., 2002; Burritt et al., 2002; Bischof et al., 2003; Contreras et al., 2009). Studies one gene expression in macroalgae demonstrated induction of genes encoding for ROS scavenging enzymes in response to high light, copper stress as well as oxidative and hyper saline stress (Collén et al., 2007; Wu and Lee, 2008; Dittami et al., 2009; Contreras-Porcia et al., 2011). We observed higher induction of transcripts associated with ROS scavenging enzymes at 2°C than at 12°C, indicating a higher oxidative stress level at low temperatures. This might be either due to enhanced generation of ROS at low temperatures, or to decreasing rate of ROS scavenging with declining temperatures. Interestingly most ROS scavenging enzymes were induced in field sporophytes of *S. latissima* subjected to UVR at 12°C, whereas only one up-regulated transcript was detected after this treatment in cultured material. This might be reflecting lower oxidative stress levels in cultured material at 12°C. The low growth temperature of field sporophytes of *S. latissima* in Spitsbergen might led to metabolic alterations, which allow a better performance of stress response at lower temperatures compared to field material, but at the same time to a higher sensitivity towards increasing temperatures.

CONCLUSION

In the present study we investigated interactive effect of UV radiation and temperature on gene expression in cultivated and field sporophytes of *S. latissima*. We detected large differences in the number of regulated genes between field and cultured material. The higher amount of regulated genes in cultivated material suggests that cultivated sporophytes undergo a larger scale metabolic reorganization for acclimating to UVR and changes in temperature than field sporophytes. Gene expression profiles revealed that DNA and photosynthesis are prime targets of UVR. Origin of the sporophytes had no influence on maximum quantum

yield of photosystem II, observed differences in gene expression of photosynthetic components under UVR of *S. latissima* might be caused by differences in age-dependent morpho-functional features. Main effects of UVR, targeting mostly photosynthesis and DNA, were similar in cultured and field sporophytes, which show that laboratory experiments are well suited to investigate basic molecular mechanisms of acclimation to abiotic stresses. The higher induction rates of transcripts associated with DNA repair and replication as well as ROS scavenging indicate a higher sensitivity to UVR and a higher oxidative stress level at 12°C in field compared to cultivated sporophytes. At the same time cultivated sporophytes grown at 10 °C must make stronger efforts of acclimating to UVR at 2°C than field sporophytes, which were exposed to low temperatures for about half a year. This is reflected by the finding that at 12°C the amount of regulated genes was only half as much compared to the 2°C and 7°C treatment in cultured sporophytes. These results demonstrate the influence of growth conditions on the acclimation to stress on the transcriptional level. We suggest that acclimation of *S. latissima* from the field to low temperatures caused metabolic alterations to increase stress performance at low temperatures, and concurrently led to higher susceptibility at 12°C. The observed interactive effect of UVR and temperature demonstrates the importance of multifactorial experiments. Our results furthermore underscore the importance of conducting experiments with field material, when predicting biological and environmental effects of changing abiotic factors in the field.

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TABLES

Table 1. Numbers of over-represented Gene Ontology terms within the three GO root categories cellular component, molecular function, and biological process among the different treatments.

Origin	Treatments	Regulation	GO root category		
			Cellular component	Molecular function	Biological process
Culture	***2°C LPAB***	***up***	18	27	13
	2°C LPAB	***down***	-	3	11
	7°C LPAB	***up***	18	11	10
	7°C LPAB	***down***	20	3	19
	12°C LPAB	***up***	8	1	2
Field	***2°C LPAB***	***up***	14	3	5
	2°C LPAB	***down***	-	1	-
	7°C LPAB	***up***	9	4	6
	12°C LPAB	***up***	15	6	4

Table 2. Enriched metabolic KEGG pathways among the significantly up- and down-regulated genes in different stress conditions identified by KOBAS analysis with a hypergeometric test ($p < 0.01$).

Origin	Treatment	Regulation	KEGG pathway	KO Id
Culture	***UV 2°C***	***up***	ABC transporters	ko02010
			Glutathione metabolism	ko00480
Culture	***UV 2°C***	***down***	Galactose metabolism	ko00052
			Pentose phosphate pathway	ko00030
			Glycolysis / Gluconeogenesis	ko00010
			Alanine, aspartate and glutamate metabolism	ko00250
Field	***UV 2°C / 7°C***	***up***	Carotenoid biosynthesis	ko00906

Table 3. Differential regulated genes encoding for photosynthetic components, DNA repair, DNA replication, vitamin B_6 biosynthesis and ROS scavenging proteins. All displayed genes were differentially expressed with $p < 0.01$ and were considered to be significant differently expressed with a fold change > 2 (numbers in bold).

Contig name	Putative gene product	Fold change					
		Culture			Field		
		2°C UV	7°C UV	12°C UV	2°C UV	7°C UV	12°C UV
Photosynthetic components							
Contig02465	Cytochrome b6 complex	**2.5**	**2.2**	**2.4**	**2.5**	**2.4**	**3.2**
Contig13579	Fucoxanthin-chlorophyll a-c binding protein	**19.7**	**20.5**	1.2	**253.9**	**8.7**	**7.5**
Contig24218	Light harvesting complex protein	**-3.2**	**-4.8**	-1.1	**-3.2**	-1.2	-1.6
Contig08085	Light-harvesting complex I 21 kDa	**-3.2**	**-4.9**	-1.1	**-7.0**	-1.4	**-2.0**
Contig03513	Photosystem I reaction center subunit II	1.3	**2.4**	1.9	**2.9**	**2.3**	**2.2**
Contig02889	Photosystem II biogenesis protein psp29	**3.6**	**2.5**	1.7	**5.1**	**3.5**	**2.4**
Contig03429	Photosystem II D2 protein	**2.3**	**2.5**	**2.4**	**2.9**	**2.1**	**3.3**
Contig14092	Photosystem II protein	1.7	**2.8**	1.8	**3.0**	1.6	1.6
Contig02680	Photosystem II stability assembly factor hcf136	**2.0**	**3.5**	**2.1**	**2.8**	1.8	**2.3**
Contig05910	Thylakoid lumenal protein	1.8	**2.9**	1.7	**2.4**	**2.8**	1.8
Contig03555	Thylakoid lumenal 15 kDa protein chloroplastic	1.4	**2.6**	1.9	**2.0**	**2.3**	**2.1**
DNA repair							
Contig21643	Deoxyribodipyrimidine photolyase family	**3.4**	**3.1**	1.1	**3.5**	**2.0**	**2.2**
Contig04132	DNA repair protein uvh3	**4.2**	**3.5**	**2.6**	**2.8**	1.8	**4.7**
Contig07877	Rad51 DNA recombination repair protein	**4.4**	**4.9**	1.8	**4.5**	**3.1**	**3.1**
Contig06170	X-ray repair cross-complementing protein 6	**2.4**	**2.2**	1.7	**3.5**	**4.6**	**3.6**
DNA replication							
Contig20059	DNA gyrase subunit b	**2.3**	**3.5**	1.7	**3.1**	**2.4**	**2.3**
Contig05303	DNA polymerase I	**2.1**	**3.2**	1.3	**4.0**	**2.1**	**2.1**
Contig20298	Replication factor-a protein	**3.7**	**3.0**	**2.9**	**3.2**	**4.1**	**4.6**
Contig07889	Replication protein-a 69 kDa DNA-binding subunit	**3.1**	**3.1**	**2.6**	**2.6**	**2.8**	**3.3**
Contig13754	Replication protein-a 70 kDa DNA-binding	**3.1**	**2.9**	**3.0**	**2.8**	**3.4**	**3.4**
Contig26375	Replicative DNA helicase	1.5	**2.2**	1.7	**3.1**	**2.6**	**2.7**
Contig23469	Ribonucleoside-diphosphate reductase large chain	**3.3**	1.3	-1.6	**2.6**	**2.5**	**3.0**
Contig02084	Ribonucleoside-diphosphate reductase small chain	**7.6**	**5.6**	1.9	**4.0**	**2.7**	**3.4**

Table 3. continued

Vitamin B_6 biosynthesis							
Contig25393	Pyridoxal biosynthesis protein pdx2	**4.6**	**3.1**	**2.1**	**3.2**	**4.3**	**2.7**
Contig03286	Pyridoxamine 5 -phosphate oxidase	**4.5**	**5.6**	**2.2**	**4.0**	**2.5**	**2.7**
ROS scavengers							
Contig10729	Alternative oxidase chloroplastic	**4.9**	**4.6**	**2.2**	**3.9**	**2.7**	**2.4**
Contig06154	Dehydroascorbate reductase	**3.8**	**5.1**	1.9	**4.1**	1.7	**2.3**
Contig00051	L-ascorbate peroxidase	**3.6**	**5.0**	1.6	**7.4**	**3.4**	1.9
Contig08467	Thioredoxin reductase	**8.9**	**9.5**	1.6	**7.9**	**6.2**	**4.7**
Contig27363	Thioredoxin-like protein	**3.6**	**2.6**	**-2.4**	**7.4**	1.1	1.3

FIGURES

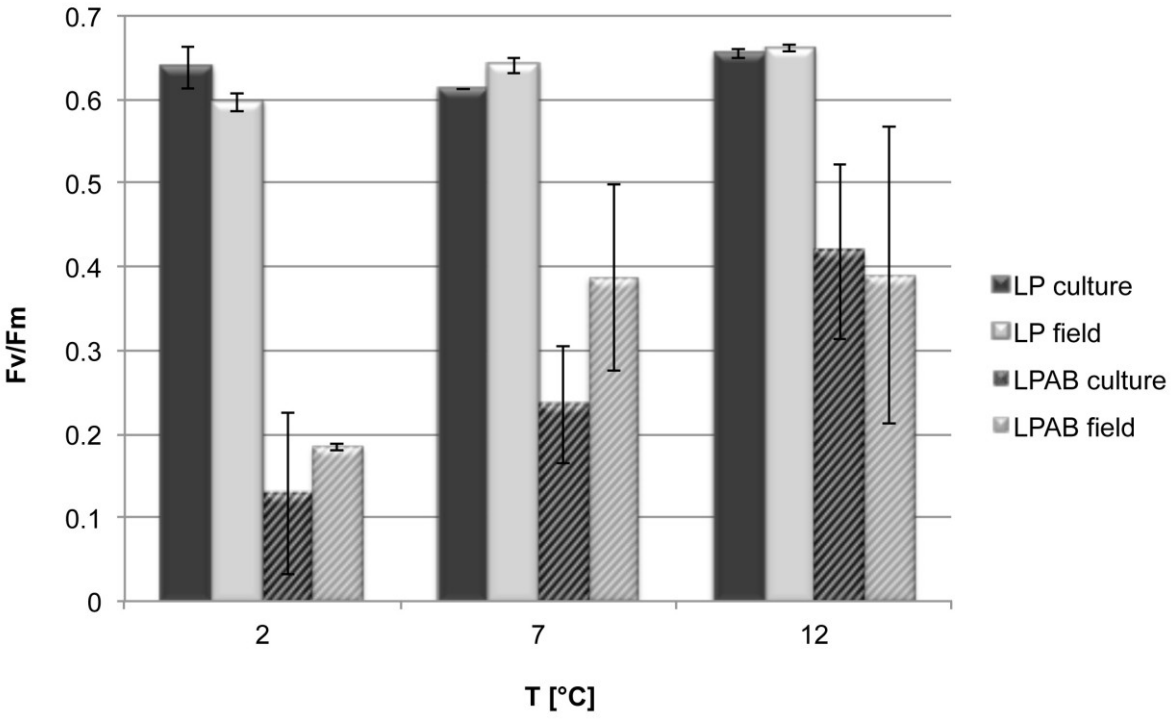

Figure 1. Maximum quantum yield of photosystem II (Fv/Fm) of *Saccharina latissima* from culture and field after 24h exposure to 2 radiation conditions [low photosynthetically active radiation (PAR) treatments = LP; low PAR+UV =LPAB] at three temperatures (2,7 & 12°C)

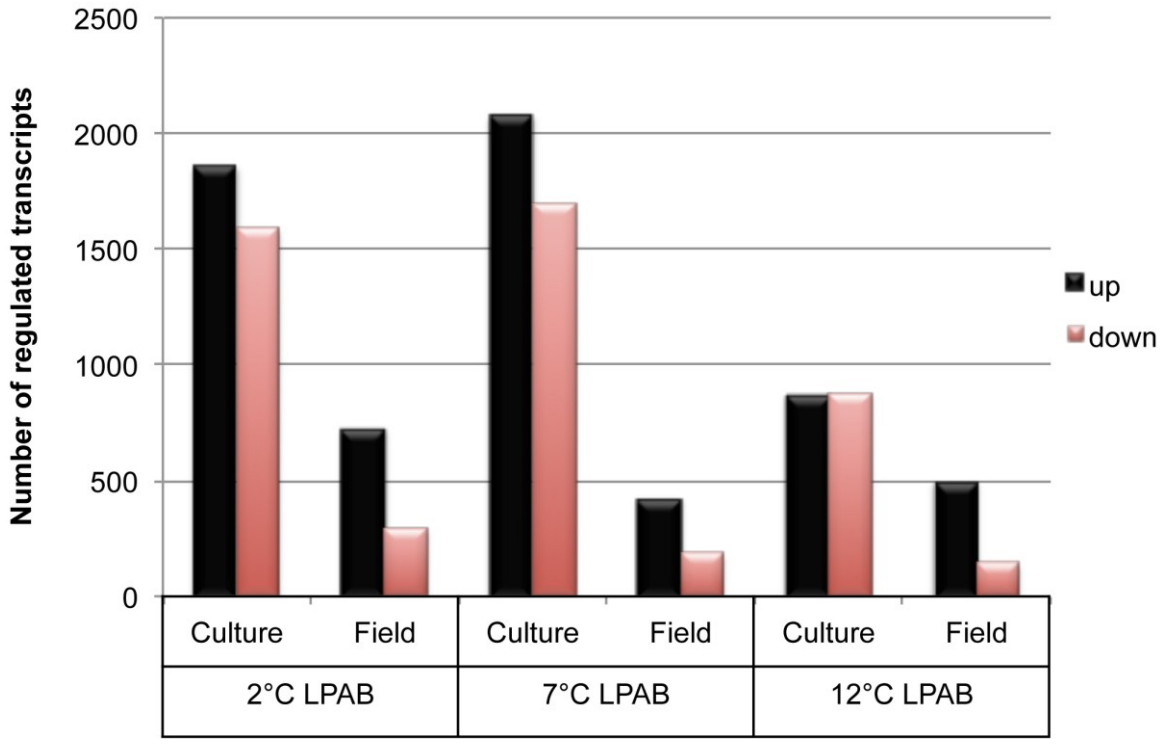

Figure 2. Number of significantly different up- (black bars) and down-regulated (red bars) genes in *Saccharina latissima* from culture and field after exposure to low photosynthetically radiation + UV (LPAB) at 3 temperatures (2, 7 & 12°C)