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Journal of Experimental Marine Biology and Ecology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Origin matters — Comparative transcriptomics in *Saccharina latissima* (Phaeophyceae)

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ARTICLE INFO

Article history:
Received 9 April 2015
Received in revised form 1 December 2015
Accepted 11 December 2015
Available online xxxx

Keywords:
Abiotic stress
Growth conditions
Interactive effects
Kelp
Saccharina latissima
Transcriptomics

ABSTRACT

Kelps, brown algae of the order *Laminariales*, dominate rocky shores of cold-temperate regions and constitute important components of coastal ecosystems. Factors influencing their distribution are light including UV-radiation, and temperature, therefore future global environmental changes will likely have an impact on their zonation, distribution patterns, and primary productivity. Here the question was addressed whether laboratory studies can allow such predictions on natural communities by exploring interactive effects of UV-radiation, temperature and growth conditions, on cultivated versus field sporophytes of *Saccharina latissima*. Both were exposed for 24 h to UV-radiation at three different temperatures (2, 7 & 12 °C), gene expression profiles under UV-radiation at different temperatures were assessed through microarray hybridizations, and comparisons of gene expression profiles in field versus culture sporophytes were carried out. Principal effects of UV-radiation were similar in culture and field sporophytes, demonstrating laboratory experiments being well suited for investigating basic molecular mechanisms of acclimation to abiotic stresses in the field. However, sporophytes from the field reacted less intense than laboratory cultures, indicating that the severity of transcriptomic responses in situ may be over-estimated from laboratory experiments.

1. Introduction

Global environmental changes, e.g. global warming and increased UV-radiation (UVR) represent major threats to polar marine ecosystems (Bartsch et al., 2012; Bischof et al., 2006; Harley et al., 2006). According to 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). Recent depletion of the stratospheric ozone layer has caused enhanced UVR at the surface, especially pronounced at high latitudes (Kerr and McElroy, 1993; Madronich et al., 1998). Also, 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 in general are endangered by global climate changes (Helmuth et al., 2006; IPCC, 2001). 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 (Carlsen et al., 2007; Lippert et al., 2001; Lüning, 1990). In addition macroalgae account for up to 10% of the global oceanic primary production (Carlsen et al., 2007; Charpy-Roubaud and Sournia, 1990; Lüning, 1990; Smith, 1981; van de Poll et al., 2003). Primary abiotic factors determining vertical zonation and geographical distribution of macroalgae are temperature and light, including UVR (Bartsch et al., 2008; Hanelt, 1998; Wiencke et al., 2006). It is thus likely that the increase of UVR 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 already been observed (Hawkins et al., 2009; Lima et al., 2007; Simkanin et al., 2005).

UVR affects negatively photosynthesis in macroalgae, e.g. leading to degradation of light harvesting complex proteins, D1/D2 heterodimer of photosystem II, and influence secondary photosynthetic reactions (Bischof et al., 2000, 2002a, 1998; Davison et al., 2007; Franklin et al., 2003). Another target of UVR in macroalgae is DNA (Britt, 1999; Hall et al., 1992; Sinha and Häder, 2002). Studies demonstrated that exposure to UVR leads to formation of cyclobutane-pyrimidine dimers (CPDs), which can be removed by photoreactivation and nucleotide excision repair (Pakker et al., 2000a; van De Poll et al., 2002). UVR susceptibility of macroalgae also seems to be life-stage dependent and is

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http://dx.doi.org/10.1016/j.jembe.2015.12.006
0022-0981/

related to their vertical distribution (Roleda et al., 2006, 2007; Wiencke et al., 2007).

While damaging effects of elevated temperatures and UVR on seaweed physiology are well studied in single factor laboratory experiments only few projects focused on interactive effects and even less effects on field grown material. 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 e.g. demonstrated interactive effects of temperature and UVR on germination of macroalgae (Hoffman et al., 2003; Müller et al., 2008). For some macroalgal species it was shown that photosynthetic efficiency under UVR was higher at moderate temperatures compared to low temperatures (Fredersdorf et al., 2009; Rautenberger and Bischof, 2006). These results underline the importance of multifactorial experiments, especially for investigating climate change effects. A perhaps even more 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 have been published, demonstrating no significant differences in physiological parameters (Bischof et al., 1999; Sagert and Schubert, 2000). Furthermore in our earlier transcriptomic studies in laboratory grown kelp we observed that treatments not causing measurable physiological reactions are yet visible in the transcriptome response to a large extent (Heinrich et al., 2012b, 2015). Such molecular response comes along with metabolic costs, which in the long run might influence growth, defense against pathogens, and further performance parameters.

Based on these findings we hypothesize that no differential physiological effects will be visible, but on the transcriptional level differences related to the origin of the sporophytes may be expected. We studied interactive effects of UVR and temperature on gene expression in *Saccharina latissima* freshly sampled in the field (Spitsbergen, Norway) versus established laboratory cultures. To prevent from population specific acclimation responses, field grown material was sampled from the same population as the established gametophytes stock cultures of *S. latissima*. The perennial kelp *S. latissima* is a common species in kelp beds of Arctic and cold-temperate coastal waters (Bolton et al., 1983; Borum et al., 2002; Gerard, 1988).

2. Material & methods

2.1. Algal material

2.1.1. Field grown material

Sporophytes of *S. latissima* with a size of 5–7 cm were collected in May 2009 directly after the sea-ice breakup by scuba diving from 8 m water depths in Kongsfjorden (N 78° 55.817′; E 011° 55.236′; 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 48 h in running seawater at 3–5 °C under low light (10 μmol photons m^{-2} s^{-1}) prior to the exposure experiments.

2.1.2. Culture material

Unialgal cultures of gametophytes of *S. latissima*, originally established in 1991 from spores of fertile sporophytes collected by SCUBA diving in Kongsfjorden (N 78° 55.817′; E 011° 55.236′; 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 cultivated at 10 ± 1 °C and 30 μmol photons m^{-2} s^{-1} white light at 18 h light:6 h dark period. Developing sporophytes were transferred after 2 weeks to aerated 5 l culture bottles and grown in PES until they reached a size of 5–7 cm.

2.2. 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 24 h to low photosynthetically active radiation (PAR) (23.6 ± 3 μmol photons m^2 s^{-1}) in combination with UVR (UV-A: 10.56 ± 1.04 W m^{-2}; UV-B: 0.45 ± 0.02 W m^{-2}). Every experiment was conducted in 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, München, Germany) for PAR treatments, and (2) 295–700 nm (URT 140 Ultraphan UV farblos, Difrega; München, Germany) for PAR + UVA + UVB treatments. PAR was provided by 3/5 Osram daylight fluorescent tubes (Biolux, 36 W; Osram, München, Germany) and measured by using a LI-250 light meter (LI-COR, Lincoln; USA). UVR was generated by 3 fluorescent tubes (UV A-340, 40 W; Q-Panel, Homestead, USA) and determined with a Solar Light PMA-2100 (Solar Light; Glenside, USA).

2.3. Fluorescence measurements

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). PAM measurements were conducted as described by Hanelt (1998). 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 statistically compared 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, Armonk, USA).

2.4. RNA-extraction and -labeling

Total RNA extractions from differently treated sporophytes were performed as described previously by Heinrich et al. (2012b) consisting of a CTAB extraction, followed by the use of a Qiagen RNA isolation kit. Total RNA was labeled with the Agilent two-color Low Input Quick Amp Labeling kit (Agilent Technologies, Waldbronn, Germany). For the pooled control sample 700 ng RNA from every low PAR treatment was mixed together prior to the labeling in equal molar concentrations. Here two pooled control samples were established, consisting of either RNA from laboratory-grown sporophytes or field grown sporophytes. RNA from stress treatments was labeled by fluorescent complementary RNA (cRNA) synthesis with cyanine-5-CTP, control sample RNA was labeled with cyanine-3-CTP. Agilent RNA Spike-In Mix (Agilent) was added to 200 ng of total RNA prior to the labeling. 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 labeled RNA was conducted following the two-color 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).

2.5. Microarray design and hybridization

Agilent's eArray online application tool was used to design 6 × 80k microarrays slides, containing 60 mer oligonucleotides probes created from a *S. latissima* cDNA library, which was established from RNA sampled under several light and temperature conditions (Heinrich et al., 2012a). The cDNA library sequence assembly was deposited at DDBJ/

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EMBL/GenBank under the accession GBBA00000000 (http://www.ncbi.nlm.nih.gov/nuccore/GBBA00000000).

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 the pooled control respective to their origin. The microarray hybridization procedure was carried out with 300 ng of cyanine-3 and cyanine-5 labeled cRNA for 17 h at 65 °C. Control/control hybridization were performed, each component of the pooled control (LP 2 °C, LP 7 °C, LP 12 °C) was hybridized against the pooled control to mitigate dye bias effects. 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. Data normalization was performed with the Agilent Feature Extraction Software version 9.1.3.1 (FE), which applies a linear normalization correction and the LOWESS smoothing. 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 EMBL-EBI; https://www.ebi.ac.uk/arrayexpress/; ID: E-MTAB-3074).

2.6. 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: An ANOVA, followed by a post hoc test Tukey HSD with the Benjamini and Hochberg (1995) FDR correction was performed. Genes were considered to be differentially expressed when the FDR corrected p-values were less than 0.01 and calculated absolute 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. Over-represented KEGG pathways were identified by KOBAS (http://kobas.cbi.pku.edu.cn/home.do) using a hypergeometric test and multiple testing corrected p of <0.01 (Benjamini and Hochberg, 1995).

3. Results

3.1. Photosynthetic measurements

Fv/Fm was similarly affected by temperature, light, as well as by interaction of light and temperature treatments, respectively in cultivated and field grown material (RM ANOVA within-subject effects: Temperature $F_{(2, 48)} = 14.736$, $p < 0.001$; light $F_{(1, 48)} = 244.725$, $p < 0.001$; temperature × light $F_{(2, 48)} = 9.864$, $p < 0.001$). Initial mean maximum quantum yield (Fv/Fm) of laboratory grown sporophytes (0.631 ± 0.03) and field grown sporophytes (0.637 ± 0.03) remained unchanged after exposure to low PAR at 2, 7, and 12 °C (Fig. 1). Exposure to low PAR + UVR significantly reduced maximum quantum yield and reduction 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). In summary laboratory and field grown material was not affected by temperature alone but by UVR alone and the combinatory effects of UVR plus temperature, whereby UVR was more harmful at lower temperatures. At the level of Fv/Fm sporophytes grown in the field could not be distinguished from cultured samples.

3.2. Microarray analysis

Gene expression profiles under UVR stress at different temperatures were obtained using oligonucleotide microarrays covering 25,262 transcripts. Fewer regulated transcripts were detected in field grown versus cultivated sporophytes in response to exposures (Fig. 2). In cultivated *S. latissima* UVR caused a changed expression of 8166 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 in a single treatment was observed after UVR exposure at 7 °C treatment when 3770 genes (15%) were affected. Furthermore strong interactive effects of temperature and UVR in laboratory grown sporophytes were observed: at 12 °C the number of regulated genes was only half of that at the 2 °C and 7 °C treatment.

In field grown material of *S. latissima* 1218 transcripts (5%) showed different expression patterns under at least one stress treatment compared to the control treatment (2, 7, 12 °C & low PAR), and highest number of regulated transcripts in a single treatment (1014 ≈ 4%) was detected after the UVR treatment at 2 °C.

For a more in depth comparison, the number of genes specifically up-regulated in field grown and cultivated *Saccharina*, respectively, were compared. Laboratory-grown sporophytes featured higher numbers of specifically regulated transcripts after all treatments (Table 1). However, in both origins no functional differences in terms of molecular processes were observed. A full list of specifically regulated transcripts in cultivated and field material including annotations can be retrieved from the supplemental material (Table S1).

3.2.1. Gene Ontology term enrichments

Gene Ontology (GO) term enrichments were applied to investigate function of significantly up- and down-regulated transcripts in different stress conditions. In cultivated sporophytes of *S. latissima* a total of 164 over-represented GO terms were detected, the highest number was found within the up-regulated genes after exposure to UVR at 2 °C (58, Table 2). In field grown sporophytes of *S. latissima* a total of 67

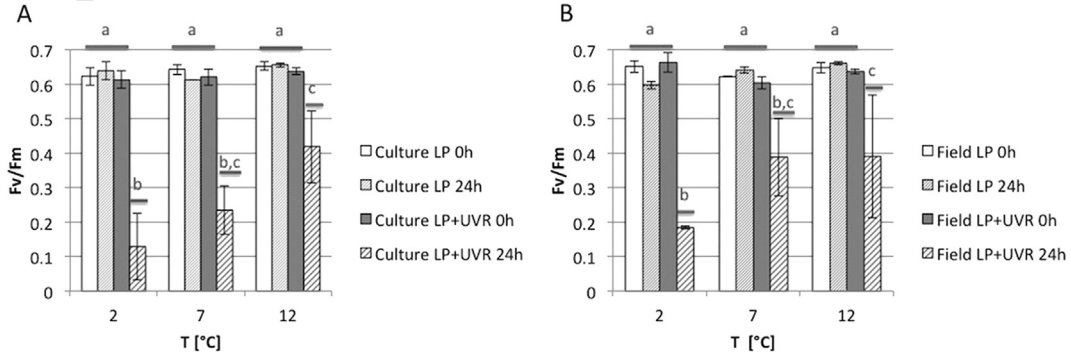

Fig. 1. Maximum quantum yield of photosystem II (Fv/Fm) of *Saccharina latissima obtained* from culture (A) and field (B) after 0 h and 24 h exposure to 2 radiation conditions [low PAR treatments = LP; low PAR + UV = LP + UVR] at three temperatures (2, 7 & 12 °C). Data are means ± SD with n = 5, significant differences are shown by alphabetic characters (post-hoc test Tukey HSD, $p < 0.01$).

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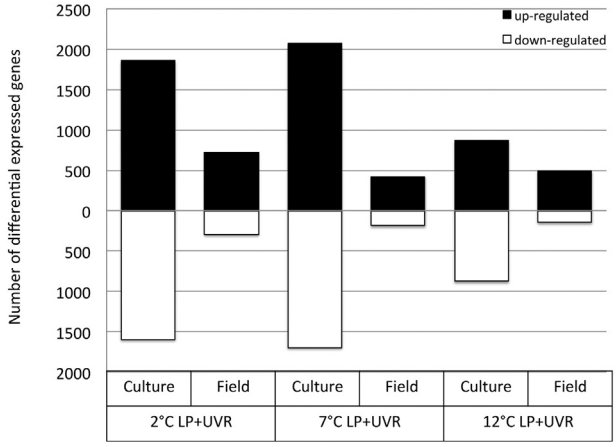

Fig. 2. Number of significantly different up- (black bars) and down-regulated (white bars) genes in *Saccharina latissima* from culture and field after exposure to low LP + UVR at 3 temperatures (2, 7 & 12 °C).

enriched GO terms was identified, with the highest number of over-represented GO terms occurring after UVR exposure at 12 °C (25, Table 1). Despite the large difference in the numbers of detected enriched GO terms in laboratory grown versus field grown material of *S. latissima*, similarity in terms of function was found. The majority of over-represented GO terms among the up-regulated transcripts of all UVR treatments were either correlated to photosynthetic components, DNA repair, or DNA replication in both sporophyte types. Cultivated sporophytes of *S. latissima* featured highest number of enriched GO terms related to nucleotides in response to UVR at 2 °C where 16 enriched terms were found; followed by the UVR conditions at 7 °C and 12 °C, with respectively 11 and 4 over-represented GO terms. In field grown material of *S. latissima* 7 over-represented GO terms correlated to nucleotides occurred after UVR exposure at 7 °C, whereas the UVR treatments at 2 °C and 12 °C featured accordingly 4 and 6 enriched GO terms. For a detailed list see Table S2.

Additional enriched GO terms were identified among the regulated transcripts in laboratory grown sporophytes versus field grown material. Induction of genes associated with vitamin metabolic processes and cellular anion homeostasis 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 and polysaccharide metabolic process.

3.2.2. KEGG pathway analyses

7 significantly enriched metabolic pathways were identified by KOBAS analysis within the UVR treatments at different temperatures (Table 3). Exposure of cultivated material to UVR 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. Field grown sporophytes under UVR at 2 °C and 7 °C increased expression of carotenoid biosynthesis.

Table 1
Numbers of transcripts specifically regulated in cultivated versus field sporophytes of *Saccharina latissima*.

Origin	Treatments	Regulation	Specifically regulated transcripts		
			Total number	Annotated	Unknown function
Culture	2 °C LP + UVR	Up	1499	499	1000
	2 °C LP + UVR	Down	1492	480	1012
	7 °C LP + UVR	Up	1879	711	1168
	7 °C LP + UVR	Down	1662	620	1042
	12 °C LP + UVR	Up	688	271	417
	12 °C LP + UVR	Down	847	197	650
Field	2 °C LP + UVR	Up	58	27	31
	2 °C LP * UVR	Down	119	29	90
	7 °C LP + UVR	Up	72	40	32
	7 °C LP + UVR	Down	124	16	108
	12 °C LP + UVR	Up	279	110	169
	12 °C LP + UVR	Down	4	1	3

3.2.3. Manual inspection of UVR-induced transcripts

GO term enrichment analysis indicated induction of transcripts correlated to photosynthetic components, DNA repair, and vitamin B_6 biosynthesis, therefore transcriptional changes of selected genes related to these categories were manually analyzed on a gene per gene base (Table 4). Furthermore the expression of reactive oxygen species (ROS) scavenging enzymes was inspected (Table 3). A full list of the regulated genes with annotations can be retrieved from the supplemental material (Table S3). Laboratory-grown sporophytes featured higher number of regulated transcripts after UVR exposure than field grown sporophytes, especially within the categories photosynthetic components, DNA repair, and ROS scavenging enzymes. To investigate whether UVR not only influences the number of regulated genes, but also the level of expression fold changes of individual genes, transcripts which were simultaneously expressed in cultivated and field material were compared.

3.2.3.1. Photosynthetic components. 9 transcripts encoding photosynthetic components significantly regulated after exposure to UVR were examined. Transcripts of the cytochrome b6 complex (contig02465) were induced in response to all treatments with transcriptional changes between 2.2 and 3.2 fold, with the highest transcript fold change in field grown sporophytes exposed to UVR at 12 °C. Regulation of three transcripts encoding for light harvesting proteins was observed, Fucoxanthin-chlorophyll a–c binding protein (contig13579, in a BLAST search most similar to the high-light LHCX clade) 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 grown material exposed to UVR at 2 °C. Light harvesting complex protein (contig24218) and light harvesting complex I 21 kDa (contig08085) were repressed in cultivated material at 2 °C and 7 °C UVR, in field grown sporophytes 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 centre subunit II featured enhanced transcript abundances in field grown sporophytes after all UVR conditions, in cultivated material only in response to UVR at 7 °C. Among 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 cultivated material exposed to UVR at 7 °C, as well as in field grown material exposed to UVR at 2 °C.

3.2.3.2. DNA repair. Several regulated transcripts associated with DNA repair were identified. Rad51 DNA recombination repair protein (contig07877) and X-ray repair cross-complementing protein 6 (contig06170) were induced in all treatments except for cultivated material subjected to UVR at 12 °C; here X-ray repair cross-complementing protein 6 showed higher fold changes in field grown sporophytes than in laboratory grown sporophytes. A deoxyribodipyrimidine photolyase family protein (contig21643) was up-regulated in all treatments except in laboratory grown 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 grown material of the UVR at 7 °C condition, highest transcript abundance in cultivated material

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Table 2
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 LP + UVR	Up	18	27	13
	2 °C LP + UVR	Down	–	3	11
	7 °C LP + UVR	Up	18	11	10
	7 °C LP + UVR	Down	20	3	19
	12 °C LP + UVR	Up	8	1	2
Field	2 °C LP + UVR	Up	14	3	5
	2 °C LP + UVR	Down	–	1	–
	7 °C LP + UVR	Up	9	4	6
	12 °C LP + UVR	Up	15	6	4

occurred after the 2 °C UVR treatment, whereas field grown sporophytes featured highest transcript abundance of this gene under UVR at 12 °C.

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 laboratory grown sporophytes after exposure to UVR at 2 °C, field grown sporophytes on the contrary showed highest transcript abundance in response to UVR at 12 °C. Furthermore fold changes of these transcripts were higher in cultivated material exposed to 2 °C UVR compared to material grown in the field.

3.2.3.3. Vitamin B_6 metabolism. Two up-regulated transcripts correlated to vitamin B_6 metabolism were detected, pyridoxal biosynthesis protein pdx2 (contig25393), and pyridoxal 5-phosphate synthase pdxh (contig03286); both were up-regulated in response to all UVR treatments. Transcript abundance at 2 °C UVR was higher in cultivated material compared to sporophytes obtained from the field, whereas at the 12 °C UVR treatment induction was stronger in field grown sporophytes than in cultivated sporophytes.

3.2.3.4. ROS scavenging enzymes. Five genes encoding for antioxidative enzymes were simultaneously regulated in cultivated as well as field grown 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 cultivated and field grown sporophytes after exposure to UVR at 2 °C. Furthermore, UVR at 2 °C and 7 °C caused stronger induction of antioxidative enzymes in cultivated material compared to the field grown material, under UVR at 12 °C sporophytes obtained from the field featured stronger up-regulation. Enhanced transcript abundance of dehydroascorbate reductase (contig06154) was observed in cultivated sporophytes after exposure to UVR at 2 °C and 7 °C, and in field grown 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 cultivated sporophytes of the UVR at 12 °C condition, with stronger up-regulation after the 2 °C/7 °C UVR treatment occurring in cultivated material than in field grown material.

Table 3
Enriched metabolic KEGG pathways among the significantly up- and down-regulated genes in different stress conditions identified by KOBAS analysis with a hypergeometric test (FDR corrected $p < 0.01$).

Origin	Treatment	Regulation	KEGG pathway	KO ID
Culture	LP + UVR 2 °C	Up	ABC transporters	ko02010
			Glutathione metabolism	ko00480
Culture	LP + UVR 2 °C	Down	Galactose metabolism	ko00052
			Pentose phosphate pathway	ko00030
			Glycolysis/gluconeogenesis	ko00010
			Alanine, aspartate and glutamate metabolism	ko00250
Field	LP + UVR 2 °C/7 °C	Up	Carotenoid biosynthesis	ko00906

4. Discussion

4.1. Photophysiological responses

Exposure to UVR in all experiments caused significant decreases in photosynthetic efficiency. Interactive effects of UVR and temperature, with the highest degree of photo inhibition occurring in sporophytes of both origins after UVR exposure at 2 °C were observed. For no treatment a significant difference between field and laboratory grown material, respectively, could be detected. Thus at the level of Fv/Fm laboratory sporophytes could not be distinguished from field grown sporophytes and the different pre-incubations of both sporophyte types do not show with this type of measurement. Our results of strong photoinhibition under UVR at low temperatures are consistent with previous studies on the interaction of UVR and temperature in macroalgae. For example 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). Sagert and Schubert (2000) investigated acclimation to light intensity of field and laboratory grown *Palmaria palmata* (Rhodophyta) and observed no differences in light saturation points (E_k) of photosynthesis. Studies on maximum quantum yield and maximum electron transport rates under UVR stress in *A. 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). It appears that maximum quantum yield and maximum electron transport rates alone are not sufficient as parameters to distinguish macroalgal laboratory strains from field grown material.

4.2. General transcriptomic response

Whereas no differences were obvious at the level of Fv/Fm between laboratory and field grown material we observed large differences in the overall number of regulated genes. From the number of reacting genes (Fig. 2) it seems as if field material needs to activate significantly only half the number of genes to acclimate. We also observed strong interactive effects of temperature and UVR on gene expression in cultivated sporophytes, at 12 °C the number of regulated genes was only half the number of the 2 °C and 7 °C treatment.

The higher number of regulated genes, enriched GO terms, and over-represented KEGG metabolic pathways found in cultivated material subjected to UVR indicates that cultivated sporophytes must make stronger efforts of acclimating to UVR than field grown sporophytes. Field plants were taken directly after the ice break-up, and we expected them therefore to be sensitive towards UVR. However we found that field grown sporophytes, based on gene and GO numbers, are less sensitive to UVR@@@ than cultivated sporophytes. One reason for this might be the possible different age of the thalli. We knew the age of laboratory sporophytes at 5–7 cm length (ca. 8 weeks) but we can only estimate the age of equally long field grown sporophytes, which could have been 4–5 months old, sporophytes obtained from the field were thicker, more stable, and appeared darker. 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 (Bischof et al., 2002b; Dring et al., 1996). For plants it was shown that exposure to a single stress agent can lead to increased resistance to subsequent unfavorable impacts (Alexieva et al., 2003). This might be also

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Table 4
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 FDR corrected $p < 0.01$ 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 LP + UVR	7 °C LP + UVR	12 °C LP + UVR	2 °C LP + UVR	7 °C LP + UVR	12 °C LP + UVR
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**
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**
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**
Contig06170	X-ray repair cross-complementing protein 6	**2.4**	**2.2**	1.7	**3.5**	**4.6**	**3.6**
Vitamin B_6 biosynthesis							
Contig25393	Pyridoxal biosynthesis protein pdx2	**4.6**	**3.1**	**2.1**	**3.2**	**4.3**	**2.7**
Contig03286	Pyridoxal 5-phosphate synthase pdxh	**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

the case for field grown sporophytes of *S. latissima*, as exposure to cold temperatures might have led to an increased resistance to UV-B radiation. It is also possible that field grown sporophytes, exposed to natural rhythms (such as day length) and depending on them, was "expecting" and pre-adapting to increased UVR along with the increased light intensity of thinning ice before break-up.

4.3. Specific transcriptional responses

Similarities of transcriptomic response to UVR in cultivated and field grown *Saccharina* include enhanced regulation of photosynthetic components and DNA repair. Several studies on UVR stress in macroalgae demonstrated that UVR influences photosynthesis negatively and causes DNA damage (Karsten et al., 2011). Our transcriptomic data support these findings, indicating that photosynthesis and DNA are main targets of UVR in macroalgae.

Even though the 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 coding for photosynthetic components, with a larger number of reacting genes in cultured sporophytes. Furthermore different expression fold changes of genes simultaneously regulated in cultivated and field grown material were observed. Transcripts encoding for light harvesting complex proteins show diverse regulation patterns in response to UVR, light harvesting complex protein and light-harvesting complex I 21 kDa showed stronger repression in sporophytes from culture while fucoxanthin-chlorophyll a–c binding protein was stronger induced in field grown sporophytes. The latter transcript was strongly up-regulated in response to the UVR treatments in both field and culture material. It was recently shown in diatoms that some members of the LHCX subfamilies of LHC genes are induced under light stress, here the authors suggested that this induction is part of a photo-protective response (Bailleul et al., 2010; Zhu and Green, 2010). The observed up-regulation of the fucoxanthin-chlorophyll a–c binding protein to UVR stress in *S. latissima* indicates a role in photo-protection. It appears that the induction of LHCs is a component of the short term and long-term acclimation of photosynthesis to both, high PAR and UVR, in *S. latissima* (Heinrich et al., 2012b, 2015). 4 genes associated with photosystem II and one gene correlated to photosystem I were detected. Photosystem I reaction centre subunit II was induced in all field grown sporophyte experiments, but only after one treatment in cultivated material, indicating a higher protein turnover rate of PS I reaction centre under UVR in field grown sporophytes compared to cultivated sporophytes. Transcripts associated to photosystem II, e.g. photosystem II D2 protein, were up-regulated in response to most of the UVR treatments, usually with higher induction in field grown 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 harmful effects of UVR than PS I (Franklin et al., 2003; Vass et al., 2005). Moreover 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 (Bischof et al., 2006; Franklin et al., 2003). For cyanobacteria it was demonstrated that repair of UVR damaged PS II reaction centre by increasing the transcript pool (Campbell et al., 1998; Huang et al., 2002). We therefore suggest that the induction of transcripts encoding for PS I, and PS II 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 cultivated and field grown 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 grown and

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cultivated sporophytes. This finding is consistent with a previous study, where it was shown that the acclimation to high irradiance increases with age of sporophytes in *S. latissima* (Hanelt et al., 1997).

UVR exposure caused in all treatments induction of transcripts correlated to DNA repair. Transcripts correlated to replication factor protein-a, e.g. replication protein-a 70 kDa DNA-binding, were induced after all UVR treatments, with highest transcript abundance in cultivated sporophytes after exposure to UVR at 2 °C, in field grown sporophytes after UVR at 12 °C. Replication protein-a binds to single-stranded DNA, 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 (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, and transcripts encoding for these proteins showed induction in response to high levels of UVR, and are therefore suggested to be required for repair of UVR-damaged DNA (Ishibashi et al., 2001, 2005). Four genes encoding for DNA repair enzymes were discovered, three of them showed enhanced transcript abundance after all UVR treatments except in cultivated sporophytes after the UVR 12 °C condition. Induction profiles of these transcripts were dependent not only 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 grown sporophytes than in cultivated sporophytes, whereas DNA repair protein uvh3 featured highest transcript abundance in cultivated material after the 2 °C UVR treatment, in field grown 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) (Britt, 1999; Hall et al., 1992). 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). A study on interactive effects of UVR and temperature on photoreactivation in *P. palmata* demonstrated that light dependent removal of CPDs increases with rising temperatures (Pakker et al., 2000b). 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 has lower kinetic rates 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 (Markmann-Mulisch et al., 2007; Sinha and Häder, 2002). Furthermore induction of DNA repair protein uvh3 was detected, a putative single-stranded DNA endonuclease involved in nucleotide excision repair of UVR- 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.

Sporophytes of both origins responded to UVR stress with an induction of the vitamin B_6 metabolism with a stronger induction of the vitamin B_6 metabolism in cultivated sporophytes compared to field grown sporophytes. Both relevant transcripts, pyridoxal 5-phosphate synthase pdxh and pyridoxal biosynthesis proteins pdx1, were most strongly induced in response to UVR at low temperatures. Vitamin B_6 acts as an important cofactor for a large number of essential enzymes, and exhibits an antioxidant activity comparable to that of vitamins C and E (Ehrenshaft et al., 1999; González et al., 2007; Leuendorf et al., 2010). Studies in *Arabidopsis* showed that vitamin B_6 is linked to stress responses, and is crucial for acclimation to oxidative, osmotic, high light and UVR stress (Chen and Xiong, 2005; González et al., 2007; Ristilä et al., 2011; Titiz et al., 2006). Vitamin B_6 deficient *Arabidopsis* plants exhibit increased sensitivity to photo-oxidative stress and the authors proposed that vitamin B6 functions in photo-protection limiting oxidative damage (Havaux et al., 2009). Our studies indicate that the regulation of the vitamin B_6 biosynthesis is a crucial part of the acclimation mechanism against abiotic stress in *S. latissima* (Heinrich et al., 2012b, 2015).

We identified several regulated genes encoding for reactive oxygen species (ROS) scavenging enzymes. UVR, and other physiological stress conditions, triggers the synthesis of ROS (Contreras et al., 2009; Kumar et al., 2010). ROS are highly toxic, cause cellular damage and need to be rapidly detoxified by cellular scavenging mechanisms (Apel and Hirt, 2004; Asada, 1997; 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 (Aguilera et al., 2002; Bischof et al., 2003; Burritt et al., 2002; Collén and Davison, 2001; Contreras et al., 2009). Stronger induction of transcripts associated with ROS scavenging enzymes at 2 °C than at 12 °C was observed, 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 kinetic rates of ROS scavenging with declining temperatures. Interestingly most ROS scavenging enzymes were induced in field grown sporophytes of *S. latissima* subjected to UVR at 12 °C, whereas only one up-regulated transcript was detected after this treatment in cultivated material. This might reflect lower oxidative stress levels in cultivated material at 12 °C. The low growth temperature of field grown sporophytes of *S. latissima* in Spitsbergen might have led to metabolic alterations, which allow for a better performance of stress response at lower temperatures, but at the same time for a higher sensitivity towards increasing temperatures.

5. Summary and conclusions

Our results indicate that cultivated sporophytes undergo a larger scale transcriptomic reorganization for acclimating to UVR and changes in temperature than field grown sporophytes. Interestingly a differentiation in terms of molecular processes was not observed here. 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 cultivated and field grown 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 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 grown sporophytes, which were exposed to low temperatures for about 4–5 months. This is reflected by the finding that at 12 °C the number of regulated genes was only half compared to the 2 °C and 7 °C treatment in cultivated 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. Our results furthermore underscore the importance of conducting experiments with field grown material for the prediction of biological and environmental effects of changing abiotic factors in the ecosystem.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jembe.2015.12.006.

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Acknowledgments

This research was funded by the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research. We would like to thank the diving group of the Alfred Wegener Institute headed by Max Schwanitz for collection of algal material. This study was performed at the Ny-Ålesund International Research and Monitoring Facility in Svalbard. [**SW**]

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Please cite this article as: Heinrich, S., et al., Origin matters — Comparative transcriptomics in Saccharina latissima (Phaeophyceae), J. Exp. Mar. Biol. Ecol. (2015), http://dx.doi.org/10.1016/j.jembe.2015.12.006

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