ON THE ROLE OF FATTY ACID COMPOSITION IN PHOTOACCLIMATION OF SEAWEEDS

Dissertation

KRISTINA KOCH

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BreMarE
Bremen Marine Ecology
Centre for Research & Education

Universität Bremen

MARINE BOTANY

On the role of fatty acid composition in photoacclimation of seaweeds

Dissertation

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by

Kristina Koch

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THESIS REVIEW

Prof. Dr. Kai Bischof, Department of Marine Botany, Bremen Marine Ecology – Centre for Research and Education (BreMarE), University of Bremen, Germany

Prof. Dr. Martin Thiel, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile

EXAMINATION COMMITTEE

Chairman:

Prof. Dr. Wilhelm Hagen, Department of Marine Zoology, Bremen Marine Ecology – Centre for Research and Education (BreMarE), University of Bremen, Germany

Additional examiners:

Prof. Dr. Kai Bischof, Department of Marine Botany, Bremen Marine Ecology – Centre for Research and Education (BreMarE), University of Bremen, Germany

Dr. Inka Bartsch, Working group Rocky Shore Ecology, Department of Functional Ecology, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany

Dr. Mirta Teichberg, Working group Algae and Seagrass Ecology, Department of Ecology, Leibniz Center for Tropical Marine Ecology (ZMT Bremen), Germany

Student members:

M.Sc. Jasmin Heiden, Ph.D. student and **B.Sc. Franziska Pausch**, Master's student of Marine Biology, Department of Marine Botany, Bremen Marine Ecology – Centre for Research and Education (BreMarE), University of Bremen, Germany and HGF Young researchers group EcoTrace, Department of Marine Biogeosciences, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany

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Dedicated to my parents, Monika and Hans-Jürgen Koch
In loving memory of my grandmother, Ursula Koch (1926-2014)

„*Zwei Dinge sind zu unserer Arbeit nötig: Unermüdliche Ausdauer und die Bereitschaft, etwas, in das man viel Zeit und Arbeit gesteckt hat, wieder wegzuwerfen.*“
Albert Einstein (1879-1955)

“*Beginnings are usually scary, and endings are usually sad, but it’s everything in between that makes it all worth living.*”
Bob Marley (1945-1981)

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SUMMARY

Throughout the world, marine macroalgae occupy highly variable habitats, in which they are exposed to various spatial and temporal gradients of environmental factors, particularly light and temperature. Variations in abiotic conditions bear the potential to cause harmful oxidative stress, if they exceed the upper or lower threshold values of tolerance. Consequently, to prevent severe stress-induced cellular damages, including chronic photoinhibition or photodamage, macroalgae rely on efficient photoacclimatory and -protective mechanisms. The importance of photosynthetic pigments (including the xanthophyll cycle), phlorotannins and antioxidants in macroalgal photoacclimation and -protection is already well established in the literature. As the photosynthetic machinery is embedded in the lipid matrix of the thylakoid membranes, the maintenance of the membrane integrity under variable environmental conditions is crucial for the physiological performance of macroalgae. However, the role of adjustments in membrane fatty acid composition is still widely understudied, although some previous studies found an highly inconsistent impact of light and temperature on the lipid profiles of macroalgae.

The overall aim of the present dissertation was to gain a deeper understanding of the role of adjustments in membrane fatty acid composition in macroalgal photoacclimation and -protection along different spatial (latitudinal and vertical) and temporal (seasonal) gradients of irradiance and temperature. In addition, other ecophysiological parameters (pigments, xanthophyll cycle, phlorotannins and antioxidants) were investigated in order to estimate the relative importance of fatty acid adjustments as photoacclimatory and -protective mechanism. For these studies, various ecologically and economically important brown and red macroalgal species were exemplarily investigated.

In **Publication I**, dealing with photoacclimatory and -protective mechanisms along a latitudinal gradient of the northern-central Chilean coast, the ecophysiological characteristics of the two brown algal species *Lessonia berteroana* (distribution: 16°S-30°S) and *Lessonia spicata* (distribution: 29°S-40°S), both belonging to the *Lessonia nigrescens* complex, were determined. In doing so, algal individuals from eight different sampling locations (27°S-32°S) were compared. Species-specific, but no site-specific differences in variable chlorophyll (Chl) *a* fluorescence of photosystem II (PSII) (photosynthetic capacity, ETR_{max} and saturating irradiance, E_k), pigments (Chl *c*, fucoxanthin and pool size of the xanthophyll cycle pigments, VAZ) and phlorotannins were found, whereas total lipid content and fatty acid composition did not differ by species or sampling location.

Publication II concentrated on acclimation processes of the brown macroalga *Macrocystis pyrifera* along a depth gradient of 4 m in northern-central Chile on various temporal scales. Different ecophysiological acclimation strategies to various water depths

between different sampling times (austral summer and winter, long-term acclimation) and more rapid adjustments to various depths during a 14-day transplantation experiment (short-term acclimation) were found. Thereby, it was shown that *M. pyrifera* adjusted its physiological state in terms of variable Chl *a* fluorescence of PSII, pigments (Chl *c*, fucoxanthin), the de-epoxidation state of the xanthophyll cycle, phlorotannins and antioxidants to the various abiotic conditions, prevailing along the vertical gradient. Moreover, modifications of total lipid contents and fatty acid profiles were found during depth acclimation, both during long-term and short-term depth exposure. Across all water depths and independently of acclimation time (long- and short-term), total lipid contents and the degree of fatty acid saturation further varied with respect to season.

In **Publication III**, discussing photoacclimation and -protection along a vertical gradient (approximately 0.5-1.0 m) on the shore of Helgoland, the ecophysiological status of the two related red macroalgae *Mastocarpus stellatus* and *Chondrus crispus* was characterized over the course of a year. Thereby, algal individuals from various positions on the shore were compared, with *C. crispus* generally occurring a bit deeper than *M. stellatus*. Species-specific differences in antioxidants, total lipids as well as the degree of fatty acid saturation and chain length were detected. Furthermore, seasonal variation was observed in total lipids and fatty acid composition of the two rhodophytes.

Overall, the findings of the three studies confirm that adjustments in photosynthetic pigments (including the xanthophyll cycle), phlorotannins and antioxidants feature a high significance in macroalgal photoacclimation and -protection. It was also found that macroalgal total lipid contents and fatty acid composition, particularly related to the degree of fatty acid saturation and chain length, clearly respond to variations in light and temperature. This might indicate that adjustments in these biochemical parameters also form part of macroalgal photoacclimatory and -protective mechanisms, exhibiting a similar significance as the other tested response variables, both on the long- and short-term time scale. Thereby, the results might denote that those adjustments not only help the algae to optimize their membrane fluidities, but also to create ideal environments for the functioning of the xanthophyll cycle under variable environmental conditions. The findings further reveal seasonal variability in fatty acids and might suggest that modulations of algal lipid profiles are negligible along latitudinal gradients, whereas they seem to play an important role in depth acclimation. Additionally, adjustments in fatty acid profiles were shown to be highly variable and species-specific, with both light and temperature having a strong impact on this response variable. The newly gained insights into photoacclimatory and -protective strategies might help to predict macroalgal responses towards challenging environmental conditions, which is crucial under ongoing climate change, and might be useful for commercial applications.

ZUSAMMENFASSUNG

Marine Makroalgen besiedeln weltweit hoch variable Habitate, in denen sie sich verändernden räumlichen und zeitlichen Umweltgradienten, insbesondere in Bezug auf Licht und Temperatur, ausgesetzt sind. Die Überschreitung spezifischer Toleranzgrenzen kann hierbei oxidativen Stress bei den Organismen auslösen. Um sich vor nachhaltigen Zellschädigungen, wie zum Bespiel chronischer Photoinhibition, zu schützen, sind Makroalgen auf effiziente Strategien der Lichtakklimatisation und des Lichtschutzes angewiesen. Es ist bekannt, dass photosynthetische Pigmente (inklusive Xanthophyllzyklus), Phlorotannine und Antioxidantien hierbei eine wichtige Rolle einnehmen. Der Photosyntheseapparat der Makroalgen ist in die Lipid-Doppelschicht der Thylakoidmembran eingebettet. Für eine konstant hohe physiologische Leistungsfähigkeit unter variablen Umweltbedingungen ist es daher von enormer Wichtigkeit für die Organismen, die Intaktheit dieser Membran aufrechtzuerhalten. Dennoch wurde die Bedeutung der Veränderung der Fettsäurezusammensetzung der Thylakoidmembran als Lichtanpassungs- und Lichtschutz-Mechanismus bisher eher vernachlässigt. Vereinzelte Untersuchungen zeigten, dass Licht- und Temperaturveränderungen einen Einfluss auf die Fettsäurezusammensetzung haben, aber diese Ergebnisse wiesen keine konsistenten Muster auf.

Die vorliegende Arbeit soll zu einem vertieften Verständnis der Funktion von Anpassungen der Fettsäurezusammensetzung als Lichtakklimatisations- und Lichtschutz-Mechanismus von Makroalgen entlang verschiedener räumlicher (latitudinal und vertikal) und zeitlicher (saisonal) Licht- und Temperaturgradienten beitragen. Darüber hinaus wurden weitere ökophysiologische Parameter (Pigmente, Xanthophyllzyklus, Phlorotannine und Antioxidantien) untersucht, um die relative Bedeutung der Fettsäurezusammensetzung für die Lichtanpassung abschätzen zu können. Für diese Studien wurden beispielhaft einige ökologisch und ökonomisch bedeutsame Braun- und Rotalgenarten ausgewählt.

Publikation I befasst sich mit den ökophysiologischen Eigenschaften hinsichtlich der Lichtanpassung und des Lichtschutzes der beiden kryptischen Braunalgenarten *Lessonia berteroana* (Verbreitung: 16°S-30°S) und *Lessonia spicata* (Verbreitung: 29°S-40°S), die zum *Lessonia nigrescens* Artenkomplex gehören, entlang eines latitudinalen Gradienten (acht Probenahmeorte: 27°S-32°S) an der Küste Nord- und Mittelchiles. Artspezifische, aber keine ortsspezifischen, Unterschiede wurden in der Chlorophyllfluoreszenz des Photosystems II (maximale Elektronentransportrate, ETR_{max} und Lichtsättigungspunkt, E_k), der Pigmentzusammensetzung (Chlorophyll *c*, Fucoxanthin und der Summe der Xanthophyllzyklus-Pigmente, VAZ) und des Phlorotanningehaltes beschrieben. Der Gesamtlipidgehalt und die Fettsäurezusammensetzung zeigten hingegen keine Unterschiede.

Sowohl die Langzeit- (jahreszeitliche Variabilität zwischen Sommer und Winter) als auch die Kurzzeit-Anpassungsfähigkeit (vierzehntägiges Transplantationsexperiment) der Braunalge *Macrocystis pyrifera* entlang eines 4m-Tiefengradienten in Nord- und Mittelchile wurde in **Publikation II** aufgezeigt. Dabei wurden Anpassungen der Chlorophyllfluoreszenz des Photosystems II, der Pigmentzusammensetzung (Chlorophyll *c* und Fucoxanthin), des De-Epoxidations-Status des Xanthophyllzyklus (DPS), der Phlorotanninkonzentration und des antioxidativen Potenzials gefunden. Zusätzlich wurden Veränderungen des Gesamtlipidgehaltes und der Fettsäurezusammensetzung entlang des Tiefengradienten, aber auch in Abhängigkeit der Jahreszeit, beobachtet.

Publikation III behandelt die ökophysiologischen Eigenschaften in Bezug auf die Lichtanpassung und des Lichtschutzes der beiden verwandten Rotalgenarten *Mastocarpus stellatus* (Verbreitung: höhere Bereiche der unteren Gezeitenzone) und *Chondrus crispus* (Verbreitung: tiefere Bereiche der unteren Gezeitenzone) entlang eines Tiefengradienten (circa 0,5-1,0 m) an der Küste von Helgoland im Jahresgang. Artspezifische und jahreszeitliche Unterschiede in dem antioxidativen Potenzial und dem Gesamtlipidgehalt, ebenso wie in dem Sättigungsgrad und der Kettenlänge der Fettsäuren wurden nachgewiesen.

Die Ergebnisse der drei Publikationen bestätigen, dass Anpassungen in Pigmenten (inklusive Xanthophyllzyklus), Phlorotanninen und Antioxidantien eine hohe Relevanz für die Lichtakklimatisation und den Lichtschutz von Makroalgen besitzen. Der Gesamtlipidgehalt und die Fettsäurezusammensetzung der Algen reagierte des Weiteren deutlich auf Licht- und Temperaturveränderungen, was darauf hindeutet, dass Anpassungen in diesen ökophysiologischen Parametern bedeutend zur kurz- und langzeitigen Lichtanpassung in Makroalgen beitragen. Diese Anpassungen scheinen dabei sowohl die Aufrechterhaltung einer optimalen Membranfluidität als auch die Optimierung der Xanthophyllzyklus-Aktivität unter variablen Umweltbedingungen zu ermöglichen. Zum einen wurde eine saisonale Variabilität der Fettsäurezusammensetzung aufgezeigt, zum anderen deuten die Ergebnisse darauf hin, dass Veränderungen in diesem Parameter bei der Anpassung entlang des latitudinalen Gradienten keine Rolle spielen, wohingegen ihnen eine hohe Bedeutsamkeit bei der Tiefenanpassung zukommt. Insgesamt waren diese Ergebnisse aber hoch variabel und artspezifisch. Die neu gewonnenen Erkenntnisse können helfen, die Reaktionen von Makroalgen auf sich verändernde Umweltbedingungen, wie zum Beispiel den Klimawandel, vorherzusagen und können für kommerzielle Anwendungen nützlich sein.

LIST OF ABBREVIATIONS

Units of abbreviated parameters are given in parentheses.

$^1O_2$	singlet oxygen
ACCase	acetyl-CoA carboxylase
acetyl-CoA	acetyl-coenzyme A
ATP	adenosine triphosphate
AWI	Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research
BEDIM	Biology, Ecology & Diversity of Invertebrates from Marine Environments
BreMarE	Bremen Marine Ecology – Centre for Research and Education
C14+C16/C18+C20	shorter-chain/longer-chain fatty acid ratio
CEAZA	Centro de Estudios Avanzados en Zonas Áridas
Chl *a*	chlorophyll *a*
Chl *c*	chlorophyll *c*
Chon-ov	*Chondrus crispus* from overlapping zone in deeper levels of the lower intertidal
CIDTA	Centro de Investigación y Desarrollo Tecnológico en Algas
CO_2	carbon dioxide
CONICYT	Comisión Nacional de Investigación Científica y Tecnológica
-COOH	carboxyl group
DGDG	digalactosyldiacylglycerol
DNA	deoxyribonucleic acid
DPS	de-epoxidation state of the xanthophyll cycle pigments
E_k	saturating irradiance of photosynthesis (µmol photons m^{-2} s^{-1})
ENSO	El Niño Southern Oscillation
ESMOI	Nucleus Ecology and Sustainable Management of Oceanic Island
ETR_{max}	maximum electron transport rate (photosynthetic capacity; µmol electrons m^{-2} s^{-1})
F_0	dark-adapted initial minimal chlorophyll fluorescence
FAS	fatty acid synthase
F_m	maximal chlorophyll fluorescence
FONDECYT	Fondo Nacional de Desarrollo Científico y Tecnológico
FS	Forschungsschiff
F_v	variable chlorophyll fluorescence
F_v/F_m	maximum quantum yield of PSII in dark-adapted state
GHI	global horizontal irradiance (MJ m^{-2})
H_2O_2	hydrogen peroxide
HCO_3^-	hydrogen carbonate
HPLC	high performance liquid chromatography
malonyl-CoA	malonyl-coenzyme A
Mast-ex	*Mastocarpus stellatus* from monospecific zone in higher levels of the lower intertidal
Mast-ov	*Mastocarpus stellatus* from overlapping zone in deeper levels of the lower intertidal
MGDG	monogalactosyldiacylglycerol
MPI	Max-Planck-Institut

MUFA	monounsaturated fatty acids
$NADP^+$	nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
NPQ	non-photochemical quenching of Chl *a* fluorescence
O_2	molecular oxygen
$O_2^{\bullet -}$	superoxide anion
$OH^{\bullet}$	hydroxyl radical
P_{680}	Chl *a* molecules in reaction center of PSII
P_{700}	Chl *a* molecules in reaction center of PSI
PAM	pulse amplitude modulation
PAR	photosynthetically active radiation (400-700 nm; µmol photons m^{-2} s^{-1})
P-E curve	photosynthesis versus irradiance curve
PG	phosphatidylglycerol
PGAL	glyceraldehyde 3-phosphate
PSI	photosystem I
PSII	photosystem II
PUFA	polyunsaturated fatty acids
Q_{10}	temperature coefficient: change of a metabolic rate due to a 10°C increase in temperature
RNA	ribonucleic acid
ROS	reactive oxygen species
RuBisCO	ribulose-1,5-bisphosphate carboxylase/oxygenase
SFA/UFA	saturated/unsaturated fatty acid ratio
SOD	superoxide dismutase
SQDG	sulfoquinovosyldiacylglycerol
SST	sea surface temperature (°C)
TAG	triacylglycerol
UACh	Universidad Austral de Chile
UCN	Universidad Católica del Norte
UCSC	Universidad Católica de la Santísima Concepción
UV	ultraviolet radiation (W m^{-2})
UV-A	ultraviolet A radiation (320-400 nm; W m^{-2})
UV-B	ultraviolet B radiation (280-320 nm; W m^{-2})
VAZ	xanthophyll cycle pigment pool
VDE	violaxanthin de-epoxidase
ZMT	Leibniz Center for Tropical Marine Ecology

1 INTRODUCTION

1.1 Marine macroalgae

Marine macroalgae, also termed seaweeds, are a large and heterogeneous group of macroscopic, multicellular algal species, conducting oxygenic photosynthesis (Hurd et al. 2014). In total, there are approximately 10000 macroalgal species, which can taxonomically be divided into the following three groups: green (Chlorophyta), brown (Phaeophyceae) and red algae (Rhodophyta; Guiry 2012). This division is originally based on differences in photosynthetic pigment composition, but further research showed that the groups also differ in a multitude of other ecological and physiological characteristics, like morphology, reproduction and biochemical composition (reviewed in van den Hoek 1982, 1996, Hurd et al. 2014).

Marine macroalgae show a broad latitudinal distribution from polar to tropical regions, where they grow predominantly in intertidal and subtidal habitats on hard bottom substrates of rocky shores (vertical zonation; Lüning 1990). There, macroalgae and particularly kelps (large brown algae, primarily of the order Laminariales) are considered as ecologically important key organisms (Steneck et al. 2002). Although macroalgae cover only 0.1% of the world's sea bottom, together with seagrasses, they account for about 5% of the total oceanic primary production (Smith 1981). In some coastal ecosystems, such as coral reefs or kelp forests (areas of large spatial dimension, which are densely populated by individuals of the brown algal genera *Ecklonia*, *Laminaria*, *Lessonia*, *Macrocystis* or *Nereocystis*; Steneck et al. 2002), their primary production even contributes to approximately 90% of total carbon fixation (Gattuso et al. 2006). Besides their high primary production rates, macroalgae are of enormous significance to coastal ecosystems by providing complex three-dimensional habitats, food sources, shelter and nursery grounds for associated invertebrates and fishes (Buschmann 1990, Schultze et al. 1990, Bartsch et al. 2008) as well as by diminishing coastal erosion through the reduction of wave power (Jackson and Winant 1983, Duggins et al. 1990). In particular, these ecosystem services are of high significance in kelp forests (Steneck et al. 2002). Furthermore, marine macroalgae are of increasing economic importance as food with benefits for humans (e.g., vitamins, minerals, long-chain polyunsaturated fatty acids (PUFA)), animal diet, source for phycocolloids (e.g., alginates, agars, carageenans), fertilizer in the agriculture, bioabsorbers in industrial and agricultural waste waters and biofuel as well as in cosmetics and pharmaceuticals (Buchholz et al. 2012). Additionally, the beauty and diversity of kelp forests attracts recreational uses (Schiel and Foster 2015).

1.2 Photosynthesis in macroalgae

As marine macroalgae are primary producers, their photosynthetic performance is the major determinant, which drives their growth and survival and thereby also the complex ecology of rocky shore ecosystems.

1.2.1 Primary and secondary reactions of photosynthesis

The multitude of processes that occur during photosynthesis in higher plants and algae can be divided into two major steps: primary reactions (energy transduction) and secondary reactions (carbon fixation, Calvin cycle). The primary reactions take place on the thylakoid membrane, whereas the secondary reactions are located in the stroma of the chloroplasts (Raven et al. 2005).

The primary reactions are catalyzed by the four integral membrane protein complexes photosystem I and II (PSI and PSII), cytochrome b6f complex and adenosine triphosphate (ATP) synthase, which are embedded in the thylakoid membrane (Figure 1.1). Each photosystem is composed of a reaction center, surrounded by an antenna complex (light-harvesting complex). Each reaction center is further made up of a dimer of chlorophyll (Chl) *a* molecules (P_{700} in PSI, with maximum light absorption at a wavelength of 700 nm and P_{680} in PSII, with a maximum absorption at 680 nm) and proteins, (e.g., D1 protein in PSII), which hold the Chl *a* molecules in place. The antenna complex consists of various accessory pigments, like chlorophylls, carotenoids and phycobilins and serves for light trapping and to broaden the range of wavelengths that can be used for photosynthesis. If algae are exposed to solar radiation, light energy is absorbed by pigments of the antenna complex and is shuttled from one pigment molecule to the next by resonance energy transfer until the Chl *a* molecules in the core of the reaction center are reached. There, an electron of the Chl *a* molecules is excited and transferred to the next electron acceptor molecule to initiate the electron flow. The cytochrome b6f complex represents a central part of this electron transport chain, in that it couples the electron transfer from PSII to PSI to the pumping of protons into the thylakoid lumen. During this transport, the electron is finally transferred to nicotinamide adenine dinucleotide phosphate ($NADP^+$, oxidized form), which is thereby reduced to NADPH (reduced form of $NADP^+$). The electron transport along the thylakoid membrane resulted in a deficit of electrons in the reaction center of PSII, which is replaced by electrons extracted from water. This reaction produces molecular oxygen (O_2) and leads to a further accumulation of protons in the thylakoid lumen. The resulting proton gradient is capable of driving generation of ATP via the ATP synthase. Finally, during the primary reactions of photosynthesis, thus, O_2, ATP and NADPH are produced (Raven et al. 2005).

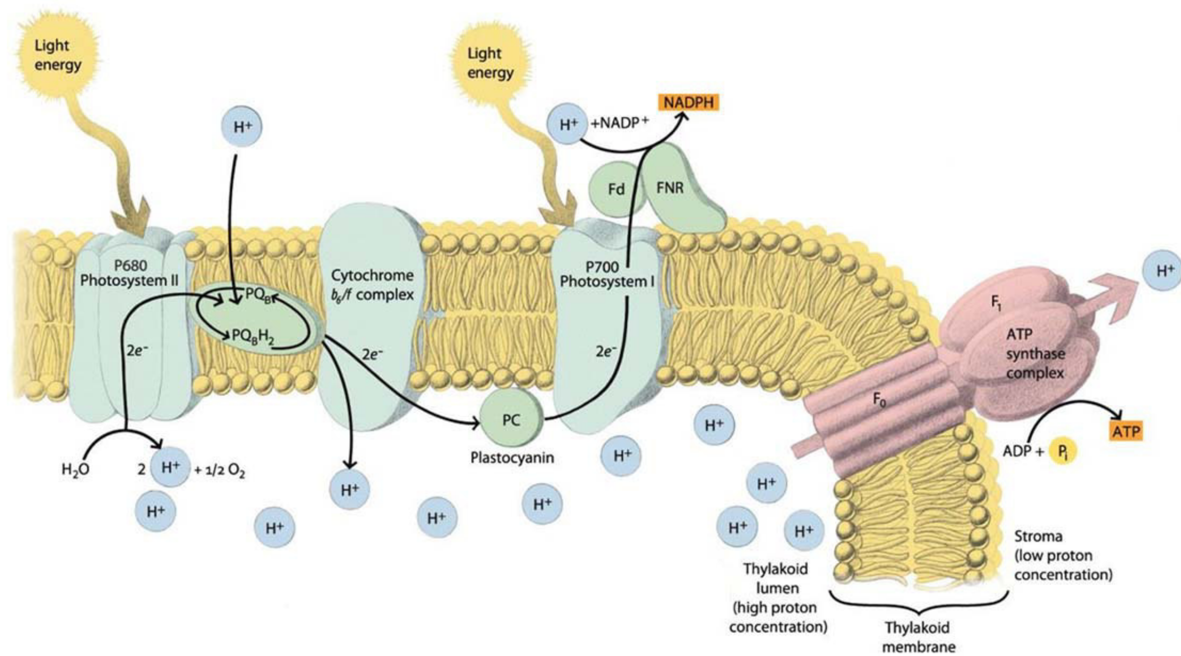

Figure 1.1: Primary reactions of photosynthesis at the thylakoid membrane. Fd, ferredoxin; FNR, ferredoxin-NADP reductase; PQ, plastoquinone (modified according to Raven et al. 2005).

In the subsequent Calvin cycle, NADPH and ATP generated during the primary reactions are used for carbon fixation via the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and formation of glyceraldehyde 3-phosphate (PGAL), the basic building block from which algae can produce a variety of substances, like glucose, starch or cellulose (Raven et al. 2005).

1.2.2 Influence of light and temperature on photosynthesis

The photosynthetic performance of macroalgae is influenced by numerous environmental parameters. Among these, light and temperature have been identified as key factors, both strongly influencing algal physiology and thereby also their geographic distribution (e.g., Hutchins 1947, Breeman 1988, Broitman et al. 2001). Therefore, the present work focused mainly on these two abiotic parameters.

The primary reactions are mainly affected by variations in light intensity. The relationship between photosynthesis and irradiance can be described by means of a P-E curve (photosynthesis versus irradiance curve; Figure 1.2). At low light intensities, the photosynthetic rate is thought to increase proportionally to the intensity (Long et al. 1994, Hurd et al. 2014). Colombo-Pallotta et al. (2006), for example, showed that blades of the giant kelp *Macrocystis pyrifera* exhibit greater net photosynthetic rates, when they grow at lower water depths and consequently higher light regimes, compared to those existing in deeper waters. If other environmental factors (e.g., temperature or carbon dioxide (CO_2) concentration) become limited, a further rise of the light intensity may result in a continuously

slower increase of the photosynthetic rate, until a plateau is reached. At very high light intensities, the photosynthetic apparatus can even be damaged, so that the photosynthetic rate decreases again (Long et al. 1994, Hurd et al. 2014).

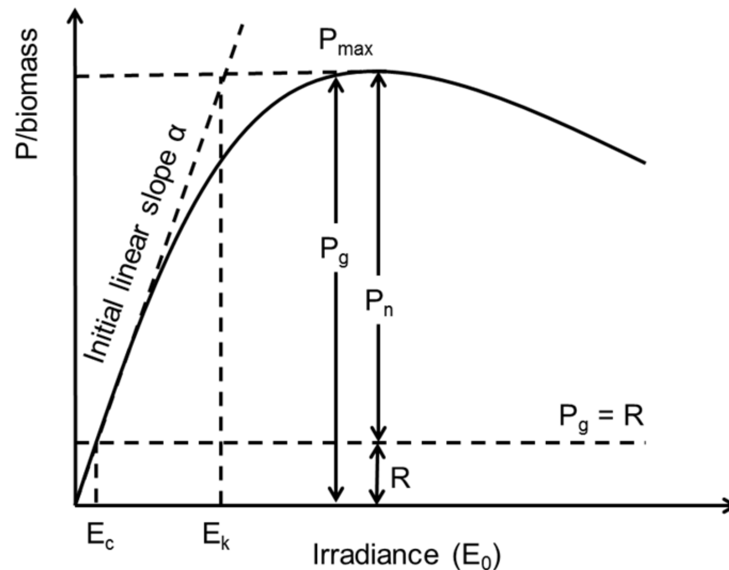

Figure 1.2: Generalized model of a P-E curve for net photosynthesis (P) versus incident irradiance (E_0). In extremely dim light, respiration (R) is greater than photosynthesis. When gross photosynthesis (P_g) balances respiration, the level of irradiance is at the compensation irradiance (E_c). The photosynthetic rate increases linearly at first, with the initial linear slope (α) describing the photosynthetic efficiency. At higher irradiances, photosynthesis becomes saturated (P_{max}), with the saturating irradiance level (E_k) being defined as the point at which the extrapolated initial linear slope α crosses P_{max}. At extremely high irradiances, photoinhibition may occur. P_n, net photosynthesis (modified according to Hurd et al. 2014).

The secondary reactions, in contrast, are influenced by changes in temperature, because these reactions are catalyzed by enzymes. The relationship between photosynthesis and temperature can be described by means of an optimum curve. It is assumed that under saturating light intensities, the photosynthetic rate doubles with every 10°C increase in temperature (Q_{10} temperature coefficient = 2). Above the species-specific optimum temperature range, the rate decreases again, since the enzymes start to denature (Lüning et al. 1990).

1.3 Lipids and fatty acids in macroalgae

Lipids are hydrophobic or amphipathic substances, being poorly soluble in water. Based on their physical characteristics, they are grouped into neutral (e.g., triacylglycerols) and polar lipids (e.g., phospho-, galacto- and sphingolipids; Buchanan et al. 2000, Raven et al. 2005).

1.3.1 Structure and biosynthesis of fatty acids

Fatty acids are carboxylic acids consisting of an aliphatic hydrocarbon chain of an even number of carbon atoms and a terminal carboxyl group (-COOH). Their physical nature is determined by the length of the hydrocarbon chain (chain length) and the extent to which the carbon atoms in this chain are saturated or unsaturated (state or degree of saturation). Saturated fatty acids are characterized by the absence of double bonds between carbon atoms (e.g., fatty acid 16:0, palmitic acid; Figure 1.3a). Unsaturated fatty acids, in contrast, possess one (monounsaturated, e.g., fatty acid 18:1(n-9), oleic acid; Figure 1.3b) or more (polyunsaturated, e.g., fatty acid 20:4(n-6), arachidonic acid; Figure 1.3c) double bonds between the carbon atoms. Double bonds induce kinks in the hydrocarbon chain, which prevent close packing among fatty acid molecules and, in turn, lowers their melting points (Buchanan et al. 2000, Raven et al. 2005).

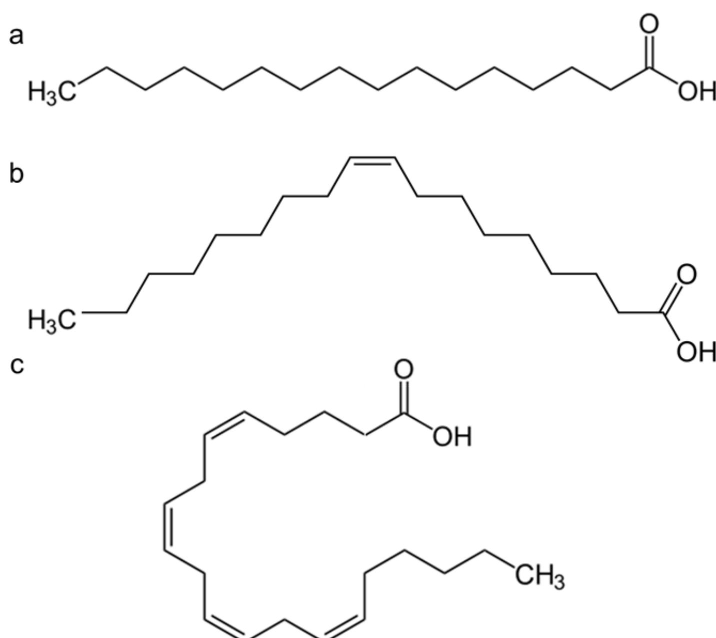

Figure 1.3: Structural formula of the **(a)** saturated fatty acid 16:0, palmitic acid, **(b)** monounsaturated fatty acid 18:1(n-9), oleic acid and **(c)** polyunsaturated fatty acid 20:4(n-6), arachidonic acid (modified according to Buchanan et al. 2000).

Most of the information on fatty acid biosynthesis in photoautotrophs is derived from investigations on higher plants and microalgae (Ohlrogge and Browse 1995). Fatty acids are synthesized almost entirely in the plastids of plants or algae, which is different from the formation of fatty acids in fungi and animals, primarily taking place in the cytosol (Thompson 1996). The fatty acid synthesis is catalyzed by the enzymes acetyl-CoA carboxylase (ACCase) and fatty acid synthase (FAS), a complex of several individual enzymes. It starts with the ATP-dependent formation of malonyl-coenzyme A (malonyl-CoA) from acetyl-coenzyme A (acetyl-CoA) and CO_2, derived from hydrogen carbonate (HCO_3^-). The thereby

produced malonyl-CoA is the central carbon donor for all subsequent reactions of fatty acid synthesis. In total, at least 30 enzymatic reactions are followed, including elongation, condensation, dehydration and reduction steps, to produce a fatty acid with 16 or 18 carbon atoms (palmitic or stearic acid; Ohlrogge and Browse 1995, Guschina and Harwood 2006).

1.3.2 Functions of lipids

In general, there are almost no free fatty acids in algal cells. Fatty acids are rather used to form more complex lipids, which occupy important functions. On one hand, polar lipids (e.g., phospholipids, but also galactolipids) are structural components of biological membranes and on the other hand, neutral lipids (e.g., triacylglycerides) function as energy-storage compounds (Ohlrogge and Browse 1995). Nelson et al. (2002) observed in a comparative study on lipid composition that polar lipids represent by far the dominant lipid class (44-94% of total lipids) in green, brown and red algae. This finding may indicate that the majority of algal lipids is structurally bound in membranes and only a much smaller proportion is functioning as storage compounds.

Phospholipids are composed of two fatty acid molecules bounded to a glycerol molecule, whose third carbon atom is linked to a phosphate group of a phosphate-containing molecule (Figure 1.4; Raven et al. 2005). This combination of a nonpolar fatty acid tail and a polar head group leads to the amphipathic characteristics of phospholipids, which are essential for the formation of membranes. The majority of biological membranes is composed of a lipid bilayer, because the amphipathic lipids, as one of their major constituents, tend to arrange themselves in this formation. Thereby, the polar head groups of the lipids are oriented towards the watery interior of the cell and the nonpolar fatty acid tails are directed towards one another (Gorter and Grendel 1925). Peripheral and integral membrane proteins (e.g., protein complexes involved in primary reactions of photosynthesis) as well as oligosaccharides are further associated with the lipid bilayer (Buchanan et al. 2000).

Figure 1.4: Structural formula of a phospholipid molecule (http://www2.chemie.uni-erlangen.de).

According to the fluid mosaic model, almost all molecules of the membrane are able to diffuse freely within the plane of the membrane, allowing membrane molecules to re-arrange rapidly and, hence, shape alterations of the membrane (Singer and Nicolson 1972).

Furthermore, biological membranes exhibit a selective permeability to various substances, so that enclosed compartments inside the cell can be formed, which are characterized by defined chemical conditions, differing from those in the surrounding. Both features permit biomembranes to be the site of important biological processes, such as signal transduction, transport of substances or maintenance of electro-chemical proton gradients and energy production via the photosynthetic electron transport chain (Buchanan et al. 2000). Each membrane of an algal cell has a characteristic lipid composition, with every single lipid molecule exhibiting a distinct fatty acid composition (Ohlrogge and Browse 1995). Most biomembranes are predominantly built up by phospholipids, but the thylakoid membranes of plants and algae contain primarily galactolipids (Quinn and Williams 1983, Klyachko-Gurvich et al. 1999, Lee 2000). Generally, about 50% of the total lipid content of thylakoid membranes is monogalactosyldiacylglycerol (MGDG) and about 30% is digalactosyldiacylglycerol (DGDG). The sulfolipid sulfoquinovosyldiacylglycerol (SQDG, approximately 5-12% of total lipid content) and the phospholipid phosphatidylglycerol (PG, approximately 5-12% of total lipid content) as well as other lipid classes make up only much smaller proportions (Figure 1.5; Lee 2000, Loll et al. 2007, Mizusawa and Wada 2012).

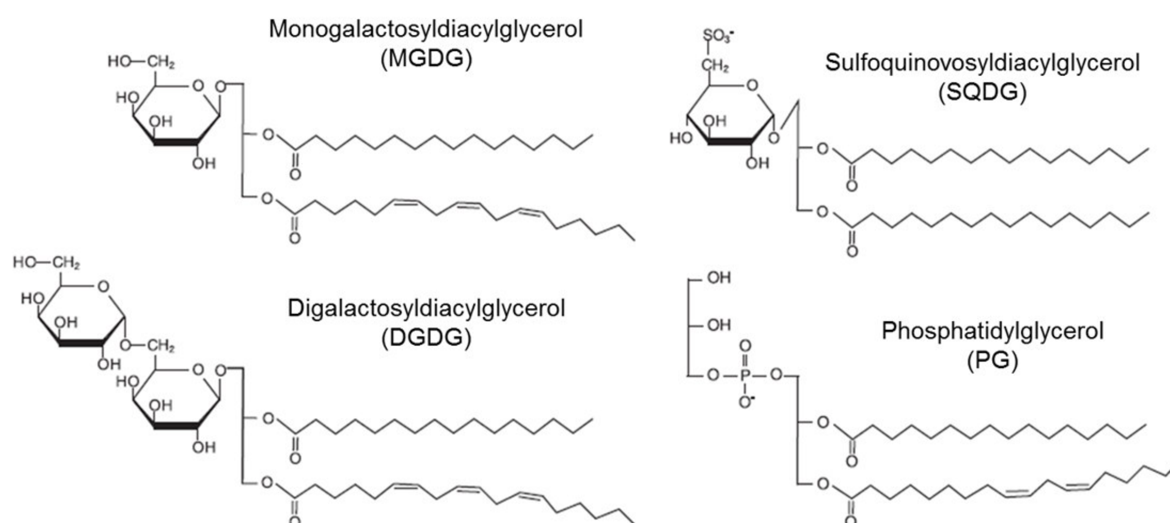

Figure 1.5: Structural formula of a monogalactosyldiacylglycerol (MGDG) molecule, a digalactosyldiacylglycerol (DGDG) molecule, a sulfoquinovosyldiacylglycerol (SQDG) molecule and a phosphatidylglycerol (PG) molecule (modified according to Mizusawa and Wada 2012).

Triacylglycerides consist of three fatty acid molecules linked to a glycerol molecule (Figure 1.6; Raven et al. 2005). This lipid class cannot work as building blocks of biological membranes, since it exhibits only nonpolar characteristics. Although only being present in low amounts in algae, triacylglycerides can serve as excellent storage compounds of chemical energy due to their higher proportion of energy-rich carbon-hydrogen bonds compared to those of carbohydrates (Ohlrogge and Browse 1995).

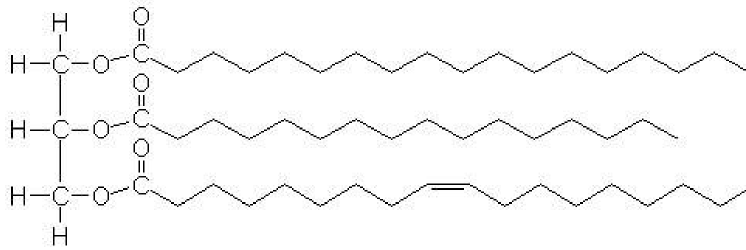

Figure 1.6: Structural formula of a triacylglyceride molecule (http://www2.chemie.uni-erlangen.de).

1.3.3 Fatty acid profiles of macroalgae

Some studies have investigated the fatty acid composition of various macroalgal species. Their results suggested evidence that the fatty acid composition is strongly linked to the taxonomic classification of macroalgae (e.g., Fleurence et al. 1994, Graeve et al. 2002, Khotimchenko et al. 2002, Hanson et al. 2010, Galloway et al. 2012). Khotimchenko et al. (2002) showed that those taxonomic differences are independent of the geographical source of the tested algae. It was proposed that rather variations in the environmental conditions of the algal habitats were responsible for the observed differences. In general, representatives of all three algal phyla are rich in fatty acid 16:0. Moreover, red algae exhibit high contents of the long-chain fatty acids 20:4(n-6) and 20:5(n-3) (eicosapentaenoic acid), whereas PUFAs with 18 carbon atoms are only present as minor components. Some red algal species also contain large amounts of fatty acid 16:1(n-7) (palmitoleic acid) and/or 18:1(n-9). Green algae, in contrast, have high concentrations of PUFAs with 18 carbon atoms, like fatty acid 18:2(n-6) (linoleic acid) and 18:3(n-3) (α-linoleic acid). Besides great levels of fatty acid 18:1(n-9), brown algae are rich in polyenoic fatty acids with 18 or 20 carbon atoms of the n-3 and n-6 series, such as fatty acid 18:2(n-6), 18:3(n-3), 18:4(n-3) (stearidonic acid), 20:4(n-6) and 20:5(n-3) (Fleurence et al. 1994, Graeve et al. 2002, Khotimchenko et al. 2002). Graeve et al. (2002) suggested that the clear differences in the fatty acid profiles between the three macroalgal phyla are probably due to their different evolutionary history. While red algae are assumed to represent the phylogenetically oldest lineage, and green algae are considered as most “modern” group, which is closely related to the higher plants, brown algae occupy the position in between. However, besides these phylum-specific fatty acid profiles of macroalgae, also discrepancies for related or even the same algal species were observed, e.g., for members of the genera *Laminaria* and *Undaria* (Vaskovsky et al. 1996, Khotimchenko 1998) and for *Gracilaria* species (Araki et al. 1990, Khotimchenko et al. 1991). It is suggested that this variance in fatty acid composition may be caused by the usage of algal samples for fatty acid analysis, which originated from various thallus parts or were collected under different environmental conditions (Westermeier and Gómez 1996, Khotimchenko et al. 2002).

1.4 Macroalgae in variable environments

Marine macroalgae live in habitats, which are characterized by very different environmental conditions. Major environmental factors affecting macroalgae are, for example, light, temperature, salinity and nutrient availability (e.g., Hutchins 1947, Breeman 1988, Broitman et al. 2001).

1.4.1 Latitudinal gradients of light and temperature

Solar radiation and sea surface temperature (SST) varies considerably on a geographic scale, with both parameters showing a general increasing trend with decreasing latitude (Broitman et al. 2001). For example, the global horizontal irradiance (GHI, 2003-2012) tends to increase along the Chilean coast from 40°S to 20°S by approximately 60% (Explorador de energía solar, http://walker.dgf.uchile.cl/Explorador/Solar2/). Sea surface temperature generally ranges from 0°C towards the poles to almost 28°C in the tropics (Figure 1.7; Hurd et al. 2014). However, local hydrodynamic features, such as upwelling of cold nutrient-rich subsurface waters or current systems, may further modulate temperature conditions on a smaller scale (Thiel et al. 2007, Tapia et al. 2014). In addition, many abiotic parameters covary with temperature. An example is nitrogenous nutrients, which are inversely related to temperature in temperate marine environments (Ladah and Zertuche-González 2007).

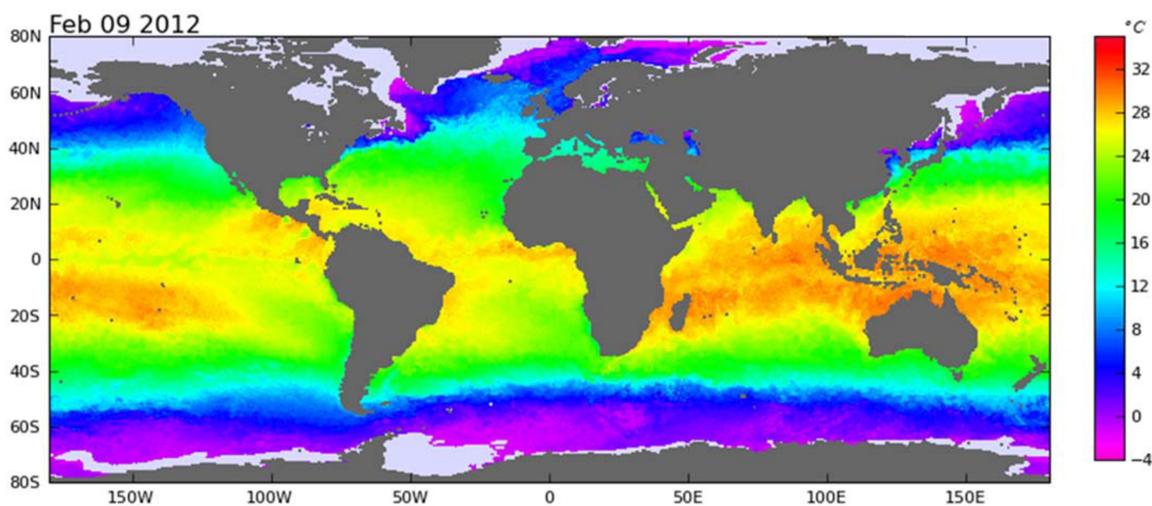

Figure 1.7: Map of global sea surface temperature, monitored on February 09, 2012. This data set was produced at 1-km (also known as ultra-high resolution) by the NASA JPL ROMS (Jet Propulsion Laboratory, Regional Ocean Modeling System) group (http://ourocean.jpl.nasa.gov).

1.4.2 Vertical gradients of light and temperature

Solar radiation reaching the Earth's surface can be classified into ultraviolet A and B (UV-A and UV-B, 320-400 nm and 280-320 nm wavelength, respectively), photosynthetically active (PAR, 400-700 nm) and infrared radiation (700-3000 nm; Figure 1.8; Lüning 1990). If solar radiation hits the water surface, it is partly reflected, depending on the angle of the sun and

the state of the surface (smooth or wavy). Reflection from a smooth surface with the sun near its zenith is below 5%, whereas with a sun altitude of 5°, reflection increases to above 40%. The presence of whitecaps and bubbles in a rough sea does further strongly enhance the proportion of reflection. The remaining light that penetrates the water surface is refracted because it travels with a slower speed in water compared to air. Additionally, it is diminished by absorption and scattering at particles, such as phytoplankton or detritus. Thereby, scattering does not directly attenuate light, but it increases the optical path of light and consequently also its opportunity for absorption. Since coastal areas are often enriched with particles, for example by inflow of sediment-loaded freshwater, the maximal depth of light penetration is considerably lower in these zones compared to open ocean areas. Generally, in clear oceanic waters light can penetrate from the surface to maximal 150-200 m depth, where only 1% of the incoming light at the surface remained (euphotic zone). However, within this depth range, each wavelength of light is not attenuated continuously, but shows a spectral change with depth. First, the infrared, red, orange and yellow ranges (longer wavelengths) as well as the UV range of the light spectrum are absorbed, followed by the green and blue ranges (shorter wavelengths), with blue light being able to penetrate deepest into the water column. Therefore, oceanic waters appear blue. In particle-rich coastal waters, in contrast, the blue range of the light spectrum is rapidly attenuated by scattering, whereas the green range dominates in the deeper water layers. Thus, coastal waters often appear green (Jerlov 1976, Lüning 1990, Kirk 2010, Hurd et al. 2014).

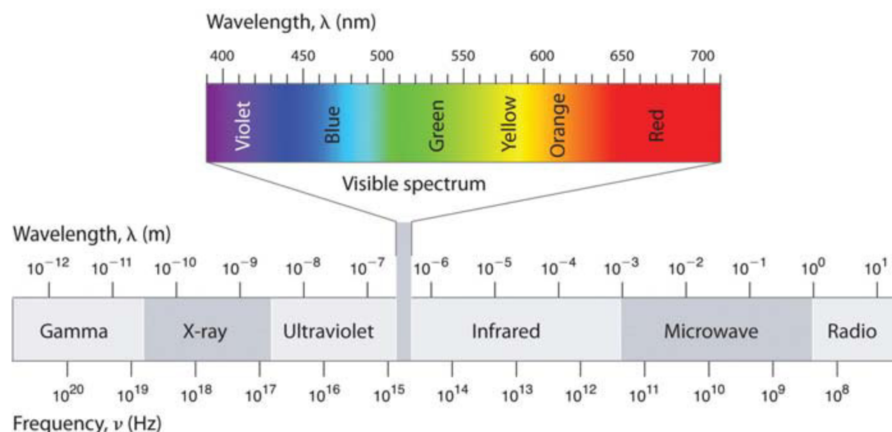

Figure 1.8: Electromagnetic spectrum of solar radiation (http://2012books.lardbucket.org/books/principles-of-general-chemistry-v1.0).

In certain coastal areas, the underwater light climate can be severely influenced by the existence of macroalgae, particularly within kelp forests. Some species, like *Macrocystis pyrifera*, form dense canopies (Rothäusler et al. 2012), which reduce surface irradiance by 63-78% directly under the canopy (Huovinen and Gómez 2011) and by 99% at 20 m depth

(Dean 1985). Movements of the canopies may further result in instantaneous changes in light climate, especially in the upper water layers (Gerard 1984).

In addition to light, temperature also decreases with increasing water depth. Solar radiation can only heat the very first meters of a water body, so that warmer and consequently less dense surface layers develop. If these surface layers are not mixed well with deeper layers due to the absence of winds, sharp temperature boundaries (thermoclines) can be formed. Consequently, the water temperature is higher above, decreases rapidly within and is, therefore, considerably lower below the thermocline. This phenomenon, also known as stratification, is common in coastal waters, especially in semi-enclosed water bodies (e.g., estuaries or fjords; Hurd et al. 2014).

1.4.3 The intertidal zone

The intertidal zone has the greatest variation in environmental factors of any marine area. Twice a day, macroalgae growing in the intertidal zone are exposed to periods of immersion in seawater during high tide and exposure to air during low tide. Thereby, they are subjected to large fluctuations in levels of PAR and UV, temperature, salinity and nutrient availability (Kübler and Davison 1993). These abiotic changes all occur simultaneously on time scales of minutes with the rise and fall of the tides and the frequency and duration of emersed periods during low tide depends on the vertical position of algae on the shore. Individuals found higher on the coast are regularly exposed to high environmental fluctuations, whereas those inhabiting lower levels are surrounded by a much more stable environment (Bell 1993, Dring et al. 1996, Sagert et al. 1997, Collén and Davison 1999).

Some potentially harmful spectral ranges of solar radiation (e.g., UV) are quickly absorbed within the water column (see Chapter 1.4.2 for details), so that they become less effective to macroalgal physiology during periods of immersion. In contrast, algae are directly exposed to these spectral ranges during exposure to air (Dring et al. 2001). Furthermore, due to its high heat storage capacity, a water body shows only a minimal range of temperature change. This means, that the temperature range, to which macroalgae are exposed during immersion, is well buffered. However, intertidal algae are subjected to drastic temperature variations during exposure to aerial conditions, where changes of 10 to 20°C compared to the seawater temperature may occur (Davison and Pearson 1996). In addition, direct exposure to solar radiation and high air temperature often promotes heating and consequently also desiccation of algae in a synergistic manner (Hurd et al. 2014).

1.4.4 Temporal variability in light and temperature

Differences in solar radiation and temperature along latitudinal and vertical gradients as well as in the intertidal zone may be further impacted by temporal variability on a daily, seasonal and interannual scale.

Diurnal fluctuations in irradiance and also temperature may occur due to changes in clouds, tides, water turbidity or the angle of the sun. Seasonal fluctuations in these abiotic parameters may either be caused by changes in day length and solar angle or by unpredictable events, like increased atmospheric cloudiness and turbidity of the water column during and after storms, runoff or plankton blooms. These temporal variations are particularly noticeable in surface water layers, whereas deeper waters are much more stable (Lüning and Dring 1979). Seasonal changes in SST differ also along the latitudinal gradient, with annual temperature ranges of <2°C in tropical and polar regions and of 5-10°C in the mid-latitude regions (Hurd et al. 2014).

In the intertidal zone, the severity of fluctuations in light, temperature and other abiotic parameters depends strongly on the time of the day during which low tide occurs. During summer, environmental conditions are most extreme, when low tide coincides with high solar radiation levels around noon. During winter, particularly in cold temperate regions, the opposite is true. Then, the air temperature is coldest at night, so that algae might freeze during low tide, whereas the temperature at noon might be high enough to prevent freezing (e.g., Davison and Pearson 1996, Huovinen and Gómez 2011).

Moreover, large-scale environmental variations, like El Niño Southern Oscillation (ENSO) events (Glynn 1988, Chavez et al. 1999, Cobb et al. 2003) or the ongoing climate change may further influence the latitudinal and vertical gradient of light and temperature. Examples for the ongoing climate change are the rise in SST caused by global warming (Wiltshire et al. 2009) or the increase in solar UV radiation reaching the Earth's surface as a consequence of stratospheric ozone depletion (Solomon et al. 1986, Manney et al. 2011).

1.5 Environmental drivers and macroalgal responses

Each variation in the abiotic environment has the potential to cause stress to a given macroalgal species, if it exceeds its upper or lower threshold values of tolerance (Davison and Pearson 1996). Whether an environmental factor is resulting in stress depends on the attributes of the factor (severity, duration, frequency and interaction with other environmental factors) and also on the macroalgal characteristics (Figure 1.9; Buchanan et al. 2000)

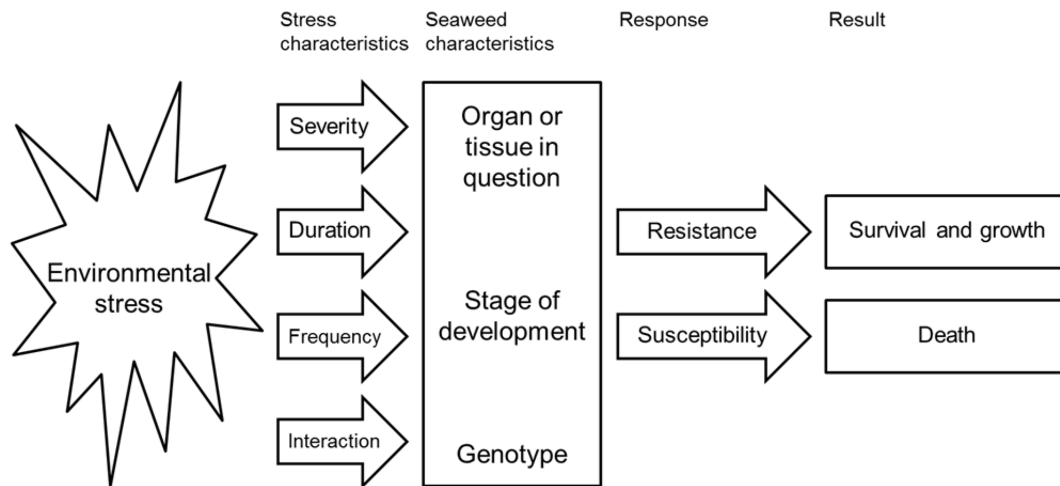

Figure 1.9: A complex interplay of external and intrinsic characteristics determines how macroalgae respond to environmental stress: the genotype and developmental circumstances of the macroalgal species, the duration and severity of the stress, the number of times a macroalgal species is exposed to stress and any additive or synergistic effects of multiple stresses. Macroalgae respond to stress through a variety of mechanisms. Failure to compensate for severe stress can result in macroalgal death (modified according to Buchanan et al. 2000).

1.5.1 What is stress?

According to Grime (1979) stress can generally be defined as "external constraints limiting the resource acquisition, growth or reproduction of organisms". Davison and Pearson (1996) divided stress further into "limitation stress", which refers to a reduction in integrative parameters, like growth, reproduction or recruitment, caused by an inadequate supply of resources (e.g., nutrients) and "disruptive stress", which includes unfavorable conditions leading to cellular damage or the need of metabolic activities to counteract or repair damage.

1.5.2 Oxidative stress

Davison and Pearson (1996) further proposed that stress should rather be defined as the response of an individual than the value of a certain environmental factor (e.g., light, temperature etc.). Oxidative stress, reflected in an increase in the level of internally generated reactive oxygen species (ROS), is generally considered as one of the major stress responses in macroalgae subjected to environmental stressors, like the exposure to high irradiances of solar radiation (PAR and UV), extremely low or high temperatures, high salinities, drought, heavy metals or air pollutants (Collén and Davison 1999, Mallick and Mohn 2000, Lohrmann et al. 2004). Reactive oxygen species are oxygen-containing molecules, which are extremely reactive due to the presence of an unpaired electron. Examples of ROS are the superoxide anion ($O_2^{\bullet -}$), the hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) (Mallick and Mohn 2000, Halliwell and Gutteridge 2015). They are able, especially in very high concentrations, to denature deoxyribonucleic acid (DNA) molecules and proteins (e.g., D1 protein) as well as peroxidase lipids of

biomembranes, with lipids containing unsaturated fatty acids being more sensitive than those containing saturated ones (Apel and Hirt 2004, Halliwell and Gutteridge 2015).

In macroalgae, the electron transport chain of photosynthesis is a prominent site for the development of ROS because of the omnipresence of O_2 (Asada and Takahashi 1987, Ledford and Niyogi 2005). Under conditions of high solar radiation, macroalgae may absorb more light energy than they can use during photosynthesis. This may result in the saturation of primary reactions and the accumulation of NADPH, which finally may restrict electron drainage. As a consequence and as a side reaction of cyclic electron transport, the so-called Mehler reaction takes place, in which electrons coming from PSI are transferred to O_2 rather than to ferredoxin, an electron acceptor, which is part of the electron transport chain. In doing so, $O_2^{\bullet-}$ is generated (Polle 1996, Asada 1999). In particular, the combination of high solar radiation and low temperature has been shown to promote the production of ROS via the Mehler reaction. Since the enzymatic steps involved in the Calvin cycle are slowed down at low temperatures, less NADPH is used up and oxidized to $NADP^+$. Consequently, this may further restrict the electron drainage and a greater amount of electrons is introduced into the Mehler reaction under these environmental conditions (Wise 1995, Lohrmann et al. 2004). Moreover, excessively absorbed light energy may lead to an overexcitation of the Chl *a* molecules located in the core of the reaction centers. Thereby, the Chl *a* molecules may turn into so-called triplet chlorophyll, which finally stimulates the formation of the extremely reactive $^1O_2$ (Asada and Takahashi 1987, Ledford and Niyogi 2005).

1.5.3 Photoacclimatory and photoprotective strategies

Macroalgae are immobile, so that they are not capable of leaving areas exposed to unfavorable environmental conditions. Thus, they are also not able to avoid the prevailing stressors and consequently the formation of ROS completely. Therefore, to prevent severe ROS-induced cellular damages, macroalgae, particularly those inhabiting the intertidal zone, rely on effective protection mechanisms to keep internal ROS levels to a minimum and hence, withstand environmental stress (Davison and Pearson 1996 and references therein).

These mechanisms can take place over different time scales. Regulation, which includes the up- and down-regulation of pre-existing enzymes, occurs within seconds to minutes. Acclimation, which involves gene expression and the synthesis of new proteins or enzymes, happens within hours to days. Adaptation, in contrast, leads to a selection of genetically determined phenotypic traits, which can result in speciation, and needs much longer time scales (thousands or even millions of years; Hurd et al. 2014).

The defense system against ROS in algae includes a multitude of enzymatic and non-enzymatic antioxidative components. Enzymes, like superoxide dismutase (SOD) or catalase work as detoxifiers of ROS and other non-enzymatic molecules, such as glutathione,

vitamins (e.g., ascorbate, α-tocopherol) or carotenoids (e.g., fucoxanthin, β-carotene), function as scavengers of ROS by mainly inhibiting the oxidation of other molecules (reviewed in Asada 1999, Mallick and Mohn 2000).

In addition to these antioxidants, macroalgae possess several other photoacclimatory and -protective mechanisms, which help them to minimize ROS formation under variable environmental conditions. Among these are adjustments in the total photosynthetic pigment pool, activation of the xanthophyll cycle or initiation of phlorotannin production.

All macroalgae can modulate the size of their photosynthetic antenna complexes in the photosystems in accordance with the prevailing light climate. Under conditions of high solar radiation, the total pigment content and the antenna sizes are reduced (Wheeler 1980, Smith and Melis 1987, Colombo-Pallotta et al. 2006, Sampath-Wiley et al. 2008, Becker et al. 2010). Smith and Melis (1987) showed, for instance, that blades of *M. pyrifera* growing in surface water layers have significantly smaller antenna sizes than those in 20 m water depth. Antenna sizes of PSI are reduced by about 50% and those of PSII even by about 90%. In doing so, the saturating irradiance of photosynthesis (E_k) is decreased and light harvesting becomes less efficient, so that the photosynthetic apparatus is protected against damage by diminishing the amount of light energy absorbed (Weykam et al. 1996, Colombo-Pallotta et al. 2006, Hanelt and Figueroa 2012).

Green and brown macroalgae are also able to dissipate excessively absorbed light energy harmlessly as heat via the so-called xanthophyll cycle. This cycle is known to be active under enhanced irradiance levels (Pfündel and Bilger 1994, Colombo-Pallotta et al. 2006) and involves the enzymatic de-epoxidation of violaxanthin to zeaxanthin through the intermediate antheraxanthin (Yamamoto et al. 1962, Demmig-Adams 1990, Pfündel and Bilger 1994).

Furthermore, exclusively brown algae exhibit inducible phlorotannins, which are phenolic compounds with several putative secondary functions. Besides their role as essential cell wall component, herbivore deterrence or antioxidant, phlorotannins are further suggested to act as sunscreen substance against potentially harmful UV radiation, due to their localization in the periphery of algal cells and maximum absorption in the UV region of the light spectrum (Pavia et al. 1997, Schoenwaelder 2002, Koivikko et al. 2005, Gómez and Huovinen 2010, Cruces et al. 2012). Since UV radiation is known to be absorbed directly by aromatic biomolecules, like DNA, ribonucleic acid (RNA) and proteins, phlorotannins are thought to play an important role in protecting brown algae against severe UV-induced cellular impairments, possibly being related to DNA replication, gene expression and protein biosynthesis (transcription, translation; Vass 1997).

1.5.4 Photoinhibition and photodamage

If macroalgae are exposed to unfavorable environmental conditions, especially high levels of irradiance, a reduction of the photosynthetic activity may occur, called photoinhibition (Powles 1984, Krause 1988, Krause and Weis 1991). According to Franklin et al. (2003), photoinhibition is defined as the failure of photoprotection to mitigate photoinactivation. For many years, photoinhibition was attributed to the light-induced damage of the photosynthetic apparatus, but more recent investigations revealed that it also includes a regulatory and protective component. Hence, Osmond (1994) divided this stress-induced decline of the photosynthetic activity into dynamic (photoprotection; Franklin et al. 2003) and chronic photoinhibition (photoinactivation).

Dynamic photoinhibition refers to all processes that decrease excitation transfer to the reaction centers causing a transient reduced photosynthetic activity, which is fully reversible on a short time period after the stress decreases again (Krause and Weis 1991). In doing so, the photosynthetic systems are protected from excess absorption of light energy (Krause and Weis 1991, Osmond 1994). The mechanisms of dynamic photoinhibition are still not entirely resolved, but the xanthophyll cycle is known to take a central role by thermal dissipation of excessively absorbed light energy (Pfündel and Bilger 1994).

Chronic photoinhibition, in contrast, is related to the inactivation and damage of the photosynthetic apparatus, which is only slowly reversible. Thereby, mainly PSII is inactivated, because its D1 reaction center protein represents a highly stress-sensitive component within the photosynthetic machinery. The recovery of the affected PSII is assured by a continuously ongoing D1 protein repair cycle, which consists of proteolytic degradation of photodamaged D1 protein and re-integration of *de novo* synthesized D1 proteins into PSII, followed by the re-activation of the reaction center. However, once the amount of absorbed light energy exceeds the capacity of photoprotection, the rate of D1 protein damage might be higher than the rate of its repair, which finally leads to a breakdown of the D1 protein pool (Mattoo et al. 1984, Ohad et al. 1984, Andersson et al. 1992, Barber and Andersson 1992, Demmig-Adams and Adams 1992, Aro et al. 1993, Park et al. 1996). Under such conditions, a great amount of the D1 protein has to be newly synthesized and PSII remains inactivated for longer time scales. These longer lasting periods of reduced photosynthetic activity may finally negatively affect macroalgal growth, reproduction and even survival (Bischof et al. 1998).

1.5.5 The role of membrane fatty acid composition in photoacclimation and photoprotection

The importance of photosynthetic pigments, the xanthophyll cycle, phlorotannins and antioxidants in photoacclimation and -protection of macroalgae is well established in the

literature (e.g., Yamamoto et al. 1962, Demmig-Adams 1990, Pfündel and Bilger 1994, Pavia et al. 1997, Asada 1999, Mallick and Mohn 2000, Schoenwaelder 2002, Koivikko et al. 2005, Gómez and Huovinen 2010, Cruces et al. 2012). However, there are only a few field studies available, targeting those parameters along gradients of environmental factors (e.g., Wheeler 1980, Smith and Melis 1987, Colombo-Pallotta et al. 2006, Sampath-Wiley et al. 2008). Moreover, it has long been overlooked and is still widely understudied whether the adjustment in biomembrane lipid composition also plays a role in photoacclimation and -protection in macroalgae.

Since PSII is embedded in the thylakoid membrane, the rate of the D1 protein repair cycle, especially the re-integration of newly synthesized proteins via lateral diffusion through the membrane, depends strongly on membrane fluidity (Ohad et al. 1984, Barber and Andersson 1992, Aro et al. 1993, Becker et al. 2010). Besides this, macroalgae have to maintain optimal membrane fluidities under variable environmental conditions to guarantee a proper operation of the photosynthetic machinery by stabilization of membrane-associated proteins as well as maintenance of electron transport chains and transmembrane proton gradients (Somerville and Browse 1991). This, in turn, strongly determines the ability of macroalgae to respond to changes in their environment and defines their survival range (Guschina and Harwood 2009).

The fluidity of a biomembrane is mainly determined by its fatty acid composition, with fatty acid chain length and degree of saturation being most crucial. It is generally accepted that at low temperatures, membranes contain higher amounts of shorter-chain and unsaturated fatty acids with lower melting points. At high temperatures, vice versa, more longer-chain and saturated fatty acids with higher melting points are incorporated into membranes. Fatty acids exhibiting lower melting points compensate for low temperature-induced decreases in membrane fluidity, whereas fatty acids possessing higher melting points increase rigidity and, thus, may prevent membrane leakage at elevated temperatures (Buchanan et al. 2000). Some previous studies have already demonstrated that changes in temperature can lead to modifications of macroalgal fatty acid profiles (e.g., Pettitt et al. 1989, Al-Hasan et al. 1991, Dawes et al. 1993, Sanina et al. 2008). Becker et al. (2010) reported, for example, that the Antarctic red alga *Palmaria decipiens* acclimated to different temperature regimes by adjusting the degree of fatty acid saturation. In addition, variations in light conditions were also shown to affect the membrane fatty acid composition of macroalgae (e.g., Pettitt and Harwood 1989, Floreto and Teshima 1998, Hotimchenko 2002, Khotimchenko and Yakovleva 2005). Since marine macroalgae are poikilothermic organisms, the sensitivity of membrane fluidity and the change in fatty acid composition in response to temperature is plausible. However, fluctuation in the fluidity is less understandable with respect to light acclimation, although it is evident that these changes in fluidity can facilitate

electron and ion transport within a membrane during photosynthesis (Klyachko-Gurvich et al. 1999). In general, the few existing investigations on the response of fatty acid composition and metabolism in macroalgae to environmental factors did not reveal consistent results. Thus, many contradictions related to changes in fatty acid composition in response to abiotic conditions and their interpretations exist. Consequently, this aspect of photoacclimation and -protection is still an important field of interest in the research of macroalgal ecophysiology.

1.6 Objectives of the thesis

As outlined above, marine macroalgae rely on a multitude of photoacclimatory and -protective mechanisms to withstand changing environmental conditions (e.g., Wheeler 1980, Colombo-Pallotta et al. 2006, Cruces et al. 2012). The fatty acid profiles of several macroalgal species have been described for taxonomic (e.g., Fleurence et al. 1994, Graeve et al. 2002, Khotimchenko et al. 2002, Galloway et al. 2012) and commercial purposes (e.g., Sánchez-Machado et al. 2004, Ortiz et al. 2009, Schmid et al. 2014), but the importance of adjustments in membrane fatty acid composition in photoacclimation and -protection has received much less attention. In particular, field studies along latitudinal, vertical and temporal gradients of various abiotic parameters are scarce. Although the few available studies showed that membrane fatty acid profiles of macroalgae change with respect to irradiance and/or temperature conditions, there are still many discrepancies in the literature (e.g., Al-Hasan et al. 1991, Dawes et al. 1993, Floreto et al. 1993, Floreto and Teshima 1998, Khotimchenko and Yakovleva 2005) Consequently, an in-depth understanding of the underlying processes is still lacking.

Thus, the overall aim of the present thesis was to gain a deeper understanding of the role of membrane fatty acid composition in macroalgal photoacclimation along different gradients of environmental factors, particularly focusing on irradiance and temperature. In addition to this aspect, other ecophysiological parameters, like photosynthetic pigments, the xanthophyll cycle, phlorotannins and antioxidants, were investigated, so that it was possible to estimate the relative importance of membrane fatty acids in photoacclimation and -protection. For these studies, various species of ecologically and economically important brown and red macroalgae were exemplarily chosen.

It was hypothesized that: **(1)** macroalgal membrane fatty acid profiles do respond to variations in environmental conditions, prevailing along different gradients. Further, it was assumed that: **(2)** adjustments in fatty acid composition are a major determinant in macroalgal photoacclimation and -protection.

In **Publication I**, the focus was set on ecophysiological acclimation mechanisms of *Lessonia berteroana* and *Lessonia spicata* (Phaeophyceae, Laminariales; Figure 1.10) along a latitudinal gradient. The two species represent ecosystem engineers (Jones et al. 1994)

and commercially important sources for alginate extraction and high-quality feed for abalone cultures (Vásquez 2008). They show broad, but contrasting latitudinal distribution ranges along the Pacific coast of South America (Tellier et al. 2009). Consequently, they experience large differences in abiotic conditions (Broitman et al. 2001, Thiel et al. 2007, Hernández et al. 2012). It was just recently discovered by Tellier et al. (2009) that *L. berteroana* and *L. spicata* are cryptic species, belonging to the species complex *Lessonia nigrescens*. Thus, only very little information about their ecology and physiology is currently available in the literature. Some previous studies revealed species-specific differences in tolerances to environmental parameters (Martínez 1999, Oppliger et al. 2011, 2012, López-Cristoffanini et al. 2013), but investigations on underlying acclimatory and -protective mechanism are completely lacking. Therefore, the present comparative approach, in which algal individuals from different locations along the coast of northern-central Chile were studied, made a first step in identifying the ecophysiological characteristics of *L. berteroana* and *L. spicata* and might help to predict the species' responses to abiotic stresses. Thereby, in particular, the question was tackled of whether potential ecophysiological differences are based on variations in genetics (species differentiation) or whether they are impacted by abiotic conditions prevailing along the latitudinal gradient.

Figure 1.10: *Lessonia nigrescens* species complex at coasts of **(a)** Mar Brava, Chiloé, Chile (http://www.algaebase.org) and **(b)** El Tabo, Chile (http://www.algaebase.org).

Publications II and **III** focused on acclimation strategies along vertical gradients of environmental parameters. Additionally, the temporal variability of these parameters was included by conducting the studies at different seasons.

In **Publication II**, acclimation processes of *Macrocystis pyrifera* (Phaeophyceae, Laminariales; Figure 1.11) to prevailing abiotic conditions at various water depths were studied in austral summer and winter in northern-central Chile. Thereby, long-term responses were referred to as consistent acclimation strategies between different seasons, whereas short-term responses were measured after a 14-day field transplantation experiment. *Macrocystis pyrifera* is considered as foundation species, as it creates a three-dimensional

habitat for associated invertebrates and fishes (Graham et al. 2007, Pérez-Matus et al. 2007, Villegas et al. 2008) and modifies underwater light (Dean 1985, Huovinen and Gómez 2011) and water motion (Jackson and Winant 1983, Duggins et al. 1990). Moreover, this kelp species shows growing economic importance, particularly in South America and southern California (Buschmann et al. 2014, Westermeier et al. 2014). *Macrocystis pyrifera* was selected for this investigation because it represents a suitable model organism for studying acclimation processes. This species reaches sizes up to 60 m, and hence, a single sporophyte may span the entire water column from the bottom to the sea surface (North 1994) and is consequently exposed to highly variable regimes of environmental conditions (North 1971, Dean 1985). Furthermore, sporophytes may become detached from the substratum, with subsequent floating to the sea surface being followed by abrupt changes in abiotic factors (Rothäusler et al. 2012). Consequently, the giant kelp must exhibit a high acclimation potential to cope with the extreme variability in environmental parameters. Some previous studies revealed that algal acclimation patterns with respect to photosynthetic performance and pigments depend on the position of the photosynthetic tissue in the water column (e.g., Wheeler 1980, Gerard 1986, Smith and Melis 1987, Colombo-Pallotta et al. 2006, García-Mendoza and Colombo-Pallotta 2007). One study showed that modifications of antioxidant and phlorotannin contents are also part of the acclimatory strategies of *M. pyrifera* (Cruces et al. 2012), but adjustments in membrane fatty acid composition has been overlooked.

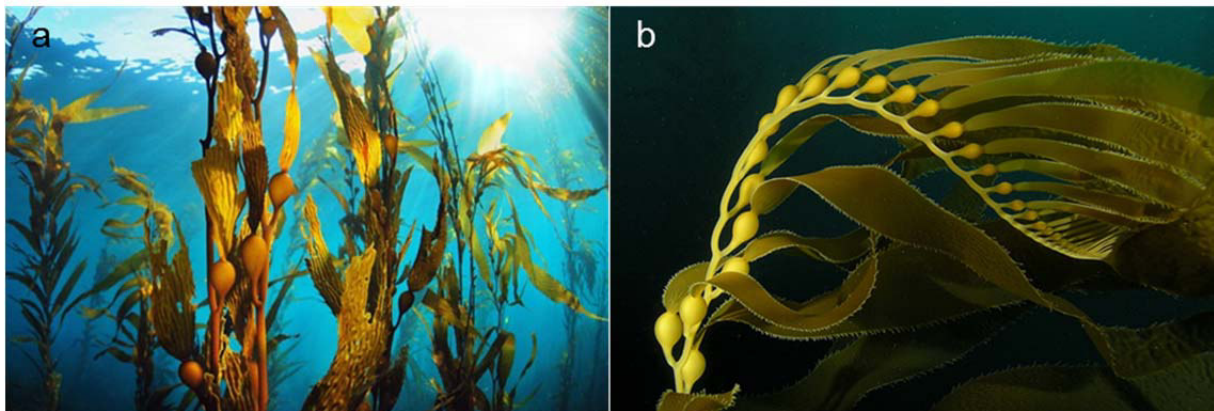

Figure 1.11: (a) *Macrocystis pyrifera* kelp forest in Tasmanian waters (http://www.seannacronin.com) and **(b)** a frond of *M. pyrifera* at a coast of Santa Barbara Island, Southern California (http://diver.net).

Publication III dealt with ecophysiological acclimation mechanism of *Mastocarpus stellatus* and *Chondrus crispus* (Rhodophyta, Gigartinales; Figure 1.12a and b) to prevailing abiotic conditions at various shore levels of the coast of Helgoland, German Bight, North Sea over the course of a year (March, May, August and October). However, compared to the vertical gradient applied in **Publication II**, the dimension of this gradient was much smaller.

Mastocarpus stellatus and *C. crispus* provide habitat and food for associated invertebrates (McLachlan 1991, Kornmann and Sahling 1994) and represent valuable sources for carrageenan (Gómez-Ordóñez et al. 2010) and PUFAs (e.g., fatty acids 20:4(n-6) and 20:5(n-3); Mabeau and Fleurence 1993). The two species were chosen for this study, because they both occupy slightly different heights of the lower intertidal zone of rocky shores along North Atlantic Ocean coastlines, with *C. crispus* generally occurring a bit deeper (Lüning 1990). Consequently, *M. stellatus* is exposed to more variable environmental conditions (Dring et al. 1996, Sagert et al. 1997, Collén and Davison 1999). Previous studies demonstrated that *M. stellatus* has a generally higher stress tolerance compared to *C. crispus* (Davison et al. 1989, Dudgeon et al. 1989, 1995, Bischof et al. 2000), which entails species-specific differences in the scavenging capacity for ROS (Collén and Davison 1999, Lohrmann et al. 2004) and UV-screening substances, like mycosporine-like amino acids (Bischof et al. 2000). Although, a few studies further pointed out that changes in irradiance and temperature can cause alterations in membrane fatty acid profiles of *C. crispus* (Pettitt et al. 1989, Pettitt and Harwood 1989), no comparative approach on differences in the fatty acid composition exists.

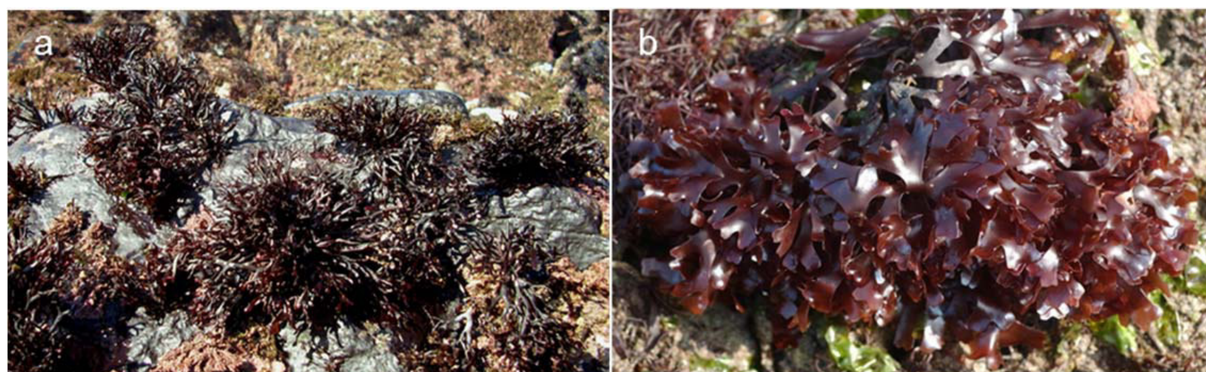

Figure 1.12: (a) *Mastocarpus stellatus* at a coast of Lugo, Galicia, Spain (http://www.algaebase.org) and **(b)** *Chondrus crispus* at a coast of New Quay, Co. Clare, Ireland (http://www.algaebase.org).

The present thesis is the first study, investigating adjustments in macroalgal membrane fatty acid compositions along various gradients of environmental factors. In doing so, new insights were added to the knowledge of photoacclimatory and -protective strategies in macroalgae. Hence, the results of the three investigations (**Publications I**, **II** and **III**) lead to a more holistic picture of acclimation to different abiotic conditions, particularly light and temperature. Such a broader understanding of photoacclimation and -protection might help to predict the responses of macroalgae towards challenging environmental conditions, which is crucial under ongoing climate change.

2 PUBLICATION OUTLINE AND DECLARATION OF CONTRIBUTION

The present dissertation is based on the following three research articles, referred to in the text by their Roman numerals.

Publication I: Species separation within the *Lessonia nigrescens* complex (Phaeophyceae, Laminariales) is mirrored by ecophysiological traits
Authors: Kristina Koch, Martin Thiel, Florence Tellier, Wilhelm Hagen, Martin Graeve, Fadia Tala, Philipp Laeseke and Kai Bischof
Journal: Botanica Marina (2015) 58(2):81-92

The experimental approach of this study was designed by K. Koch, M. Thiel and K. Bischof. Algae were sampled by K. Koch, with the assistance of members of the BEDIM laboratory. Species identification data were provided by F. Tellier. The majority of the ecophysiological analyses (measurement of Chl *a* fluorescence and total lipid contents as well as sample preparation for determination of pigment concentrations and fatty acid compositions), data evaluation and statistics was performed by K. Koch. Technical assistance helped to conduct the analysis of pigments via HPLC. Fatty acid composition data were provided by M. Graeve and phlorotannin data were gathered by P. Laeseke in the framework of his PM4 project. The manuscript was prepared by K. Koch and discussed with the co-authors.

Publication II: Short- and long-term acclimation patterns of the giant kelp *Macrocystis pyrifera* (Laminariales; Phaeophyceae) along a depth gradient
Authors: Kristina Koch, Martin Thiel, Wilhelm Hagen, Martin Graeve, Iván Gómez, David Jofre, Laurie C. Hofmann, Fadia Tala and Kai Bischof
Journal: Journal of Phycology (2016) 52:260-273

The conceptual design of this study was developed by K. Koch, M. Thiel and K. Bischof. In Jan/Feb 2012, the field work (collection of algae, transplantation experiment) and the measurement of Chl *a* fluorescence was carried out by K. Koch, with the aid of members of the BEDIM laboratory. In Aug/Sept 2012, D. Jofre was responsible for these tasks. Laboratory work (measurement of phlorotannin and total lipid contents as well as sample preparation for determination of pigment concentrations and fatty acid compositions) and the related data evaluation was done by K. Koch, with technical assistance for the HPLC measurements of pigments. M. Graeve contributed with fatty acid composition data. Antioxidant data were provided by I. Gómez. Statistical analyses were carried out by K. Koch

in close cooperation with L. Hofmann. The manuscript was written by K. Koch, with support and input from the co-authors.

Publication III: Fatty acid compositions associated with high-light tolerance in the intertidal rhodophytes *Mastocarpus stellatus* and *Chondrus crispus*
Authors: Kristina Koch, Wilhelm Hagen, Martin Graeve and Kai Bischof
Journal: Helgoland Marine Research (minor revision)

The original idea for the experimental design of this study was developed by K. Koch and K. Bischof. Algae were collected and high-light stress experiments were performed by K. Koch. Laboratory analyses (measurement of Chl *a* fluorescence and phycobilin, antioxidant and total lipid contents as well as sample preparation for determination of pigment concentrations and fatty acid compositions) were conducted by K. Koch, with technical assistance for the HPLC measurements of pigments. Fatty acid composition data were provided by M. Graeve. Data processing and statistical analyses were carried out by K. Koch. The manuscript was written by K. Koch, with revisions and improvements from the co-authors.

3 PUBLICATION I

SPECIES SEPARATION WITHIN THE *LESSONIA NIGRESCENS* COMPLEX (PHAEOPHYCEAE, LAMINARIALES) IS MIRRORED BY ECOPHYSIOLOGICAL TRAITS

Kristina Koch[1,2], Martin Thiel[3,4,5], Florence Tellier[6], Wilhelm Hagen[2,7], Martin Graeve[8], Fadia Tala[3,9], Philipp Laeseke[1,2] and Kai Bischof[1,2]

[1]Marine Botany, University of Bremen, Bremen, Germany
[2]Bremen Marine Ecology – Center for Research and Education (BreMarE), University of Bremen, Bremen, Germany
[3]Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile
[4]Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, Chile
[5]Nucleus Ecology and Sustainable Management of Oceanic Island (ESMOI), Coquimbo, Chile
[6]Departamento de Ecología, Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Concepción, Chile
[7]Marine Zoology, University of Bremen, Bremen, Germany
[8]Ecological Chemistry, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany
[9]Centro de Investigación y Desarrollo Tecnológico en Algas (CIDTA), Coquimbo, Chile

Botanica Marina (2015) 58(2):81-92

ABSTRACT

Lessonia nigrescens used to be an abundant kelp species along the Chilean coast, but recent molecular studies revealed the existence of a *L. nigrescens* species complex, which includes the two cryptic species *Lessonia berteroana* and *L. spicata*. Since these species have different distributions (16°S-30°S for *L. berteroana* and 29°S-42°S for *L. spicata*), they experience differences in environmental conditions, such as solar irradiance, seawater temperature and air exposure during low tide. This study tested to what extent the genetic distinctness of each of the two species [identified by a mitochondrial marker (*atp8/trnS*)], is reflected by ecophysiological traits (total lipids, fatty acid composition, phlorotannins, pigments and variable chlorophyll *a* fluorescence of PSII) in response to the respective environmental conditions, prevailing along the latitudinal gradient. We studied algal individuals from eight populations (27°S-32°S, including the species overlapping zone) Phlorotannins, pigments and Chl *a* fluorescence of PSII were most crucial for species-specific adaptations at the respective growth sites, whereas changes in total lipids and fatty acid compositions were negligible. Hence, species differentiation within the *L. nigrescens*

complex is also manifested at the ecophysiological level. These findings may help to predict kelp responses towards future environmental changes.

KEYWORDS

Chile, fatty acid composition, *Lessonia nigrescens* complex, phlorotannins, photosynthetic pigments

ABBREVIATIONS

α, initial linear slope of P-E curve (photosynthetic efficiency); ACE, Chañaral de Aceituno; ANOVA, analysis of variance; APO, Apolillado; β-caro, β-carotene; BSA, Bahia Salado; Chl, chlorophyll; DPS, de-epoxidation state of the xanthophyll cycle pigments; E_k, saturating irradiance; ERM, Ermitaño; ETR, electron transport rate; ETR_{max}, maximum electron transport rate (photosynthetic capacity); FAME, fatty acid methyl ester derivate; Fuc, fucoxanthin; F_v/F_m, maximum quantum yield; HPLC, high performance liquid chromatography; HSD, honest significant difference; MUFA, sum of monounsaturated fatty acids; PAM, pulse amplitude modulation; PAR, photosynthetically active radiation; PBL, Playa Blanca; PCH, Pichicuy; P-E curve, photosynthesis versus irradiance curve; PSII, photosystem II; PTAL, Punta de Talca; PUFA, sum of polyunsaturated fatty acids; ROS, reactive oxygen species; sat/unsat FA, saturated/unsaturated fatty acid ratio; SEM, standard error of the mean; SFA, sum of saturated fatty acids; SST, sea surface temperature; TEA, Punta Teatinos; UCN, Universidad Católica del Norte; UV, ultraviolet radiation; VAZ, xanthophyll cycle pigment pool

INTRODUCTION

Members of the kelp genus *Lessonia* (Bory de Saint-Vincent, 1825; Lessoniaceae, Laminariales) are distributed in the Pacific and Atlantic Oceans of the Southern Hemisphere. Along the coasts of Chile, Peru, Tasmania, New Zealand and the sub-Antarctic islands (Nelson 2005), *Lessonia* represents an ecosystem engineer (*sensu* Jones et al. 1994) and forms extensive kelp beds of significant ecological and economic importance (Steneck et al. 2002). These kelp beds exhibit high primary production rates (Tala and Edding 2007), provide three-dimensional habitats, refuge and shelter, food sources and nursery grounds for associated invertebrates and fishes (e.g., Santelices et al. 1980, Villouta and Santelices 1984), and modify water motion (Santelices and Ojeda 1984). Additionally, *Lessonia* is harvested commercially for alginate extraction and high quality feed for abalone cultures (Vásquez 2008).

Along the Pacific coast of South America, *Lessonia nigrescens* used to be one of the major representatives of the genus *Lessonia* (Searles 1978). Recently, molecular work by Tellier et al. (2009) revealed that *L. nigrescens* is actually a species complex, which includes two cryptic species (*Lessonia berteroana* Montagne (1842) and *Lessonia spicata* (Suhr) Santelices; as renamed by González et al. 2012). In their study, Tellier et al. (2009) detected that, among other characteristics, *L. berteroana* and *L. spicata* vary by the size of the intergenic spacer *atp8/trnS*, a mitochondrial marker previously used for phylogenetic studies (Voisin et al. 2005, Tellier et al. 2009). Since the size of this marker is unique for each of the two species, species identification can be performed easily via nucleotide electrophoresis, hence without sequencing (Tellier et al. 2011c).

Both cryptic species inhabit the middle to low intertidal zones of wave-exposed rocky shores (Santelices et al. 1980), but they show contrasting latitudinal distribution ranges along the southeast Pacific coast, with *L. berteroana* occurring from 16°S to 30°S and *L. spicata* from 29°S to 42°S (Tellier et al. 2009). In the zone between 29°S and 30°S, the two species spatially overlap in strict parapatry, so that a mosaic of monospecific populations either of *L. berteroana* or *L. spicata* can be observed. This strict geographic segregation of the two species is accompanied by a complete absence of interspecific gene flow and consequently the lack of hybridization (reproductive isolation; Tellier et al. 2011a). The location of the overlapping zone corresponds to a biogeographic transition zone at 30°S, which represents a margin for numerous marine organisms with low dispersal potentials (Camus 2001, Haye et al. 2014) and is characterized by changes in their recruitment patterns (e.g., of some Phaeophyceae; Meneses and Santelices 2000). Nonetheless, the distribution of species with a higher dispersal potential, such as the gastropod *Concholepas concholepas*, is less affected by the transition zone. Those species cross the transition zone without any changes in abundance (Broitman et al. 2001, Cárdenas et al. 2009). Until now the cause for this biogeographic transition zone located at 30°S is not entirely resolved, but is presumably the outcome of a combination of ancient and present day oceanographic features (e.g., breaks in eddy kinetic energy, equatorward wind stress and upwelling regimes; Hormazabal et al. 2004, Thiel et al. 2007).

Since the two cryptic species cover contrasting distribution ranges, they experience differences in environmental conditions, for example in seawater temperatures. Consequently, the species may differ in their tolerances with respect to temperature (Martínez 1999, Oppliger et al. 2011, 2012). For example, Martínez (1999) found that young sporophytes of the *L. nigrescens* complex sampled at 20°S grow better at higher temperatures (19°C, 12 days of incubation) compared to those sampled at 40°S. Similarly, gametophytes of *L. berteroana* can tolerate higher temperatures (20°C, 25 days of incubation) than those of *L. spicata* (Oppliger et al. 2012). In the latter study, further

temperature-related differences in life history strategies were detected between the species. At increased temperatures, *L. berteroana* displays a shorter haploid phase, whereas *L. spicata* shows an extended haploid phase with remarkable vegetative growth. The different temperature optima for the two species are apparently related to the species' geographic origin and play an important role in the adaptation to the prevailing local seawater temperatures. Besides genetic differences and contrasting tolerance ranges, these species vary in very few morphological characteristics. Based on the external morphology, for example, individuals of *L. spicata* are shorter and show more dichotomies than those of *L. berteroana*. Internally, blades of *L. spicata* are composed of smaller and higher amounts of cortex cells as well as more filaments in the medulla compared to those of *L. berteroana* (González et al. 2012). However, since those morphological differences are solely based on relative traits, we suggest that the two species are only completely distinguishable from each other by genetic identification, especially within the overlapping zone.

The objective of this study was to test to what extent the genetic identity of the two cryptic species within the *L. nigrescens* complex is reflected by ecophysiological traits. To check for ecophysiological differences, sporophytes of *L. berteroana* and *L. spicata* were collected at eight locations (27°S to 32°S) along the coast of northern-central Chile. Thereby, algal individuals were selected from within the overlapping zone and from within the monospecific core zone of each species. Ecophysiological differences and potential adaptive traits at their specific growth sites were addressed by measurements of total lipids, phlorotannins, pigments and variable chlorophyll (Chl) *a* fluorescence of photosystem II (PSII). Further, variations in fatty acid composition were studied for the first time. More specifically, we tackled the question of whether observed ecophysiological differences are based on variations in genetics (species differentiation, identified by a mitochondrial marker (*atp8/trnS*)) or whether they are impacted by environmental conditions prevailing along the latitudinal gradient.

MATERIALS AND METHODS

Algal material and sampling sites

Individuals of *Lessonia* spp. (without holdfast) were detached at low tide from the middle to low intertidal of rocky shores at eight locations along the coast of northern-central Chile (Figure 3.1 and Table 3.1) under comparable solar irradiance conditions during January to February 2012. At each sampling location, at least ten individuals of similar size and weight were collected in order to minimize differences in the physiological status due to size/age effects. After collection, algal individuals were kept in darkness and were immediately transported in coolers with seawater at ambient water temperature to the marine laboratory

at Universidad Católica del Norte (UCN) in Coquimbo, Chile (29°57`S, 71°20`W), where they were stored overnight in large outdoor flow-through seawater tanks (approximately 2000 l).

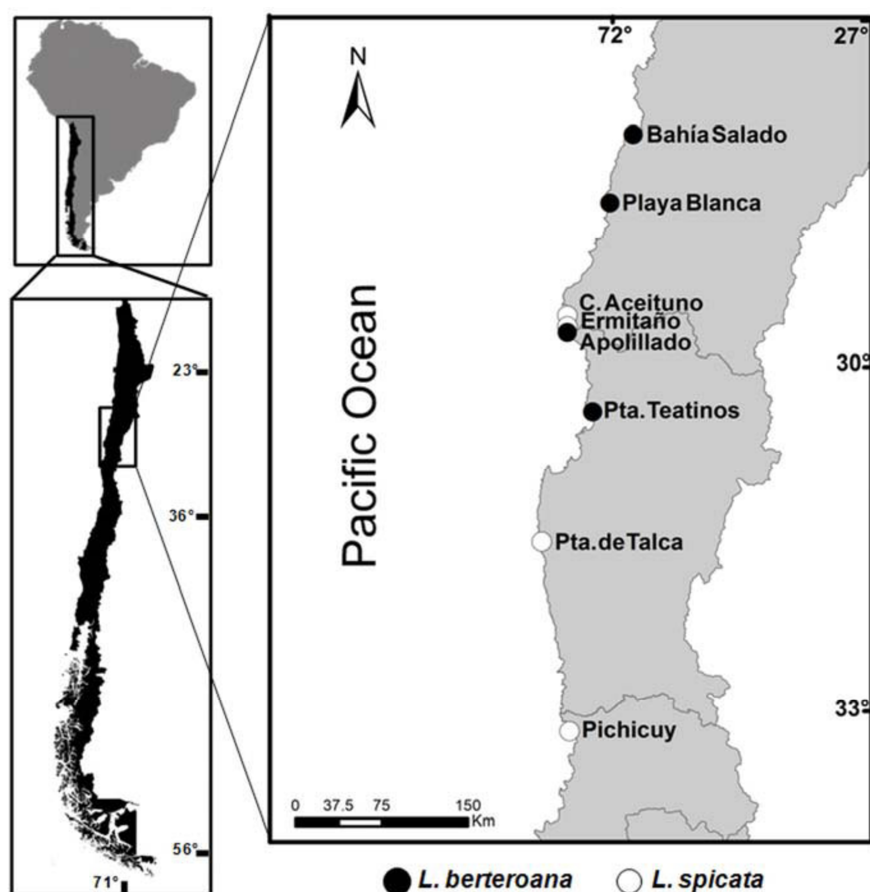

Figure 3.1: *Lessonia berteroana* and *Lessonia spicata*: map of Chile indicating the sampling sites along the northern-central coast, at which algal individuals were collected. Species were identified using the mitochondrial marker *atp8/trnS*. See Table 3.1 for further details.

Table 3.1: *Lessonia berteroana* and *Lessonia spicata*: sampling sites along the coast of northern-central Chile, at which algal individuals were collected.

Sampling site	Abbreviation	Species	Latitude (S)	Longitude (W)	Sampling date (2012)
Bahia Salado	BSA	*L. berteroana*	27°39´	70°58´	February 01
Playa Blanca	PBL	*L. berteroana*	28°11´	71°09´	February 01
Chañaral de Aceituno	ACE	*L. spicata*	29°04´	71°29´	February 21
Ermitaño	ERM	*L. spicata*	29°09´	71°29´	February 21
Apolillado	APO	*L. berteroana*	29°12´	71°29´	February 13
Punta Teatinos	TEA	*L. berteroana*	29°49´	71°17´	February 22
Punta de Talca	PTAL	*L. spicata*	30°50´	71°41´	January 26
Pichicuy	PCH	*L. spicata*	32°19´	71°28´	January 31

For each site, the abbreviated site name, geographic coordinates and sampling date are given. Species were identified using the mitochondrial marker *atp8/trnS*.

Species identification

Although the two cryptic species (*Lessonia berteroana* and *Lessonia spicata*) existing within the *Lessonia nigrescens* complex have largely disjoint geographic distributions, their distribution ranges overlap between 29°S and 30°S. Within this overlapping zone, *L. berteroana* and *L. spicata* form a mosaic of monospecific populations (Tellier et al. 2011b). In order to distinguish between the species, molecular characterization was conducted for a subset of individuals: ten individuals for the locations within the overlapping zone (ACE, ERM, APO, TEA) and five individuals for the locations located north and south of this zone (BSA, PBL, PTAL, PCH; Table 3.1).

Species identification was done using the mitochondrial intergenic spacer *atp8/trnS*, a species-diagnostic marker previously used for similar purposes (Tellier et al. 2011a). Based on the length polymorphism of this marker, species were identified through nucleotide electrophoresis and a subset of 16 individuals was sequenced to check the accuracy of the method. DNA extraction, PCR amplification, electrophoresis migration and DNA sequencing were performed according to Tellier et al. (2009, 2011c). Thereby, it was confirmed that individuals collected at the locations BSA, PBL, APO and TEA belonged to the species *L. berteroana*, whereas individuals sampled at the locations ACE, ERM, PTAL and PCH were representatives of *L. spicata* (Table 3.1).

Ecophysiological analyses

To test for differences in the ecophysiological status and potential adaptive traits at the specific growth sites of *L. berteroana* and *L. spicata*, the following response variables were measured from five individuals per sampling location: total lipid content, fatty acid composition, phlorotannin and pigment content and variable Chl *a* fluorescence. In the morning (09:00, local time), four vegetative blades of each individual were gently cleaned of epibionts. Algal disks (1.5 cm diameter, hereafter subsamples) were cut from the four blades with a cork borer and haphazardly selected for measurements of the different response variables. Measurements of variable Chl *a* fluorescence were carried out immediately, whereas subsamples for the other physiological analyses were shock-frozen in liquid nitrogen and stored at -80°C for later processing. Subsamples for species identification were dried in plastic bags filled with silica gel until DNA extraction (see above).

Total lipid content and fatty acid composition

The algal subsamples were lyophilized for 48 h and pulverized at 1500 rpm for 1 min with liquid nitrogen in a homogenizer (Mikro-Dismembrator, Typ U, B. Braun Biotech International GmbH, Melsungen, Germany). Total lipids were extracted in dichloromethane:methanol (2:1 per volume) following the methods described by Folch et al. (1957) and Bligh and Dyer

(1959). Extracts were mixed and ultrasonicated and total lipid contents were determined gravimetrically after Hagen (2000). For the analysis of fatty acid composition, aliquots of the algal extracts were taken. Fatty acids were converted to their methyl ester derivates (FAMEs) by transesterification with methanol (Merck, Darmstadt, Germany) containing 3% concentrated sulphuric acid (Merck, Darmstadt, Germany) for 4 h at 80°C. After extracting the FAMEs three times with hexane (Merck, Darmstadt, Germany), their composition was analyzed using a HP 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a DB-FFAP column (60 m length, 0.25 mm inner diameter, 0.25 µm film thickness; Agilent Technologies, Waldbronn, Germany) operated with temperature programming according to the method of Kattner and Fricke (1986). FAMEs were identified by comparing their retention times with those derived from standards of known composition. Individual fatty acids were presented as mass percentage of total fatty acids. Based on the individual fatty acid composition, they were grouped according to their degree of saturation.

Phlorotannins

The total soluble phlorotannin content was determined using the Folin-Ciocalteu method described in Cruces et al. (2012). Purified phloroglucinol (Sigma-Aldrich, Seelze, Germany) was used as standard. Algal subsamples were lyophilized for 24 h and pulverized at 4 m s^{-1} for 20 s in a high-speed benchtop homogenizer (FastPrep®-24; MP Biomedicals, Solon, OH, USA). Soluble phlorotannins from subsamples (approximately 10 mg dry weight) were extracted in 1 ml of 70% acetone (Merck, Darmstadt, Germany) for 24 h at 4°C under shaking. After centrifugation (10 min, 4°C, 2500 *g*), 50 µl of the supernatant was mixed with 250 µl of deionized water, 200 µl of 20% sodium carbonate ($NaCO_3$; Sigma-Aldrich, Steinheim, Germany) and 100 µl of 2N Folin-Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany). After 45 min of incubation at room temperature in the dark and centrifugation (3 min, room temperature, 2000 *g*), the absorbance was read at 730 nm using a microplate reader (FLUOstar OPTIMA; BMG Labtech GmbH, Ortenberg, Germany). Subsamples were measured in triplicate. Soluble phlorotannin contents were expressed as micrograms per milligram dry weight.

Pigments

Pigment determination was performed by reversed-phase HPLC. Algal subsamples were lyophilized for 24 h and pulverized at 4 m s^{-1} for 20 s in a high-speed benchtop homogenizer. Pigments from subsamples (approximately 40 mg dry weight) were extracted in 1 ml of ice-cold 90% acetone (Merck, Darmstadt, Germany) for 24 h at -20°C in the dark. After centrifugation (5 min, 4°C, 13000 *g*) and filtration through a 45-µm nylon syringe filter

(Nalgene®, Nalge Nunc International, Rochester, NY, USA), HPLC analysis was performed on a LaChromElite® system equipped with a chilled autosampler L-2200 and a DAD detector L-2450 (VWR-Hitachi International GmbH, Darmstadt, Germany). A Spherisorb® ODS-2 column (25 cm x 4.6 mm, 5 µm particle size; Waters, Milford, MA, USA) with a LiChropher® 100-RP-18 guard cartridge was used for the separation of pigments, applying a gradient according to Wright et al. (1991). Peaks were detected at 440 nm and identified as well as quantified by co-chromatography with standards for Chl *a* and *c*, fucoxanthin (Fuc), β-carotene (β-caro), violaxanthin, antheraxanthin and zeaxanthin (DHI Lab Products, Hørsholm, Denmark) using the software EZChrom Elite ver. 3.1.3. (Agilent Technologies, Santa Clara, CA, USA). Pigment contents were expressed as micrograms per milligram dry weight. The de-epoxidation state (DPS) of the xanthophyll cycle was calculated as described in Colombo-Pallotta et al. (2006).

Chl *a* fluorescence

In vivo variable Chl *a* fluorescence of PSII was measured with a pulse amplitude-modulated fluorometer (Diving-PAM; Walz, Effeltrich, Germany). The maximum quantum yield (F_v/F_m) was determined in dark-adapted (5 min) algal subsamples. Electron transport rates (ETR) were estimated from rapid photosynthesis versus irradiance curves (P-E curves). Algal samples were irradiated with a series of stepwise increasing actinic irradiances (approximately 150-2150 µmol photons m^{-2} s^{-1}) at 30-s intervals, provided by a halogen lamp (Schreiber et al. 1994). Subsequently, the photosynthetic capacity (ETR_{max}, maximum electron transport rate), the photosynthetic efficiency (α, initial linear slope) and the saturating irradiance (E_k) were defined by P-E curve fitting after Jassby and Platt (1976). We are aware that the reliability of P-E parameters derived from a Diving-PAM is hampered by potential shifts in the emission spectra during recording. However, those limitations have been accepted, as we only took account of the relative difference in the species from different sampling sites.

Statistical analysis

A one-factorial analysis of variance (one-way ANOVA) and an independent Student's t test were carried out to evaluate differences in the ecophysiological status (lipids, fatty acids, phlorotannins, pigments, variable Chl *a* fluorescence of PSII) of algal individuals between the different locations and between the algal species. When the ANOVA revealed significant differences, a post-hoc Tukey's honest significant difference (HSD) test was applied. For Student's t test comparisons along the full latitudinal range (27°39´S to 32°19´S), individual values of all four sampling sites from one species were pooled and tested against the corresponding pooled values from the other species (BSA, PBL, APO and TEA for

L. berteroana and ACE, ERM, PTAL and PCH for *L. spicata*). For Student's t test comparisons within the overlapping zone (29°04´S to 29°49´S), individual values of the two sampling sites from one species were pooled and tested against the corresponding pooled values from the other species (APO and TEA for *L. berteroana* and ACE and ERM for *L. spicata*). Prior to all statistical analyses, percentage data were arcsine-transformed. Further, all data were tested for normality and homogeneity of variances, using Kolmogorov-Smirnov's test and Levene's test, respectively. Non-normal and/or heterogeneous data were log-transformed. The software PASW Statistics 18 (SPSS; Armonk, NY, USA) was used for statistical analyses. Critical significance levels of 5% were applied.

RESULTS

Overall, total lipid contents and saturation states of fatty acids such as sum of saturated fatty acids (SFA), sum of monounsaturated fatty acids (MUFA), sum of polyunsaturated fatty acids (PUFA) and saturated/unsaturated fatty acid ratio (sat/unsat FA) of algal samples did not differ by species (Table 3.2) or sampling location (data not shown).

Table 3.2: *Lessonia berteroana* and *Lessonia spicata*: fatty acid compositions, sum of saturated fatty acids (SFA), sum of monounsaturated fatty acids (MUFA), sum of polyunsaturated fatty acids (PUFA) (mass% of total fatty acids), saturated/unsaturated fatty acid ratio (Sat/unsat FA) and total lipid content (% of dw) of algal samples collected during January to February 2012.

Fatty acid	**Species**	
	L. berteroana	***L. spicata***
14:0	4.0±0.2	4.1±0.1
16:0*	22.7±0.7	20.8±0.4
16:1(n-7)	4.3±0.2	5.1±0.1
18:0	1.1±0.2	0.9±0.1
18:1(n-9)	17.5±1.0	14.8±0.6
18:2(n-6)	6.0±0.2	6.7±0.2
18:3(n-6)	1.0±0.1	1.4±0.1
18:3(n-3)	4.6±0.3	4.3±0.2
18:4(n-3)	8.0±0.5	8.4±0.4
20:0	1.1±0.0	0.8±0.0
20:4(n-6)	18.5±0.6	20.6±0.3
20:4(n-3)	0.9±0.1	1.0±0.1
20:5(n-3)	8.0±0.5	7.7±0.4
SFA	29.6±0.8	27.5±0.5
MUFA	22.3±0.9	20.6±0.6
PUFA	48.1±1.6	51.9±1.0
Sat/unsat FA	0.4±0.0	0.4±0.0
Total lipids	2.04±0.09	1.97±0.08

The nomenclature of fatty acids (a:b(n-x)) is defined as follows: a=no. of C-atoms (chain length), b=no. of double bonds and (n-x)=position of first double bond relative to the methyl-end. For each species, data are given as pooled values from all four sampling sites (BSA, PBL, APO and TEA for *L. berteroana* and ACE, ERM, PTAL and PCH for *L. spicata*). See Table 3.1 for site name abbreviations. Table shows means±SEM (n=20). Asterisks indicate significant differences between species along the full latitudinal range (independent Student's t test, p<0.05).

In general, 13 different fatty acids (four saturated and nine unsaturated fatty acids) were detected in the algal samples (Table 3.2); 16:0 (approximately 21.8% of total fatty acids) was the most abundant saturated fatty acid and 20:4(n-6) (approximately 19.5% of total fatty acids) the dominant unsaturated fatty acid. Other principal fatty acids were 16:1(n-7), 18:1(n-9), 18:2(n-6), 18:4(n-3) and 20:5(n-3). In both species, fatty acid compositions did not show clear differences with respect to geographic latitude (data not shown); only 18:2(n-6) increased with increasing latitude (BSA: 5.7±0.1% of total fatty acids and PCH: 7.8±0.3% of total fatty acids; $p<0.001$). Fatty acid compositions also did not differ between the two species, except for 16:0. *Lessonia berteroana* exhibited significantly higher contents of 16:0 than *L. spicata*. This species-specific difference could be recognized along the full latitudinal range ($p=0.045$; Table 3.2) and within the overlapping zone (*L. berteroana*: 24.1±0.8% of total fatty acids and *L. spicata*: 21.1±0.3% of total fatty acids; $p=0.020$; data not shown).

The calculation of soluble phlorotannin contents and pigment concentrations on a dry weight and Chl *a* basis led to very similar results. Therefore, we decided to use dry weight as a reference for both physiological parameters. Along the full latitudinal range, soluble phlorotannin contents tended to be higher in *L. berteroana* compared to *L. spicata*, with the highest amounts present in individuals of *L. berteroana* from PBL and APO (42.1±6.4 and 47.0±5.8 µg mg^{-1} dw, respectively) and the lowest in *L. spicata* from ERM and PTAL (9.4±2.7 and 8.1±1.8 µg mg^{-1} dw, respectively; Figure 3.2). Due to high data variability, the t test comparison between the species did not confirm this difference as being significant. However, within the overlapping zone, *L. berteroana* had more than three times as much soluble phlorotannin as *L. spicata* ($p=0.003$).

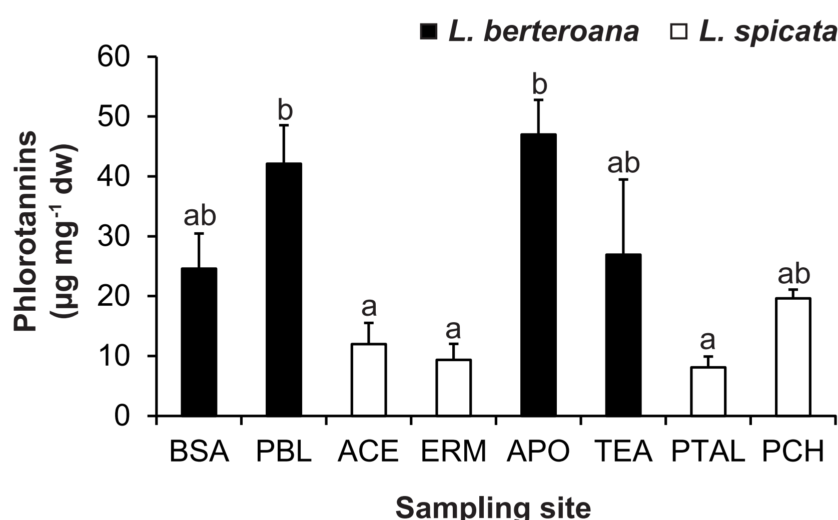

Figure 3.2: *Lessonia berteroana* and *Lessonia spicata*: soluble phlorotannin contents (µg mg^{-1} dw) of algal samples collected at eight different sampling sites during January to February 2012. See Table 3.1 for site name abbreviations. Bars are means±SEM (n=5). Different letters (a and b) indicate differences among sampling sites that are significant at $p=0.05$ (one-way ANOVA followed by a post-hoc Tukey's HSD test).

Pigment composition of algal samples did not differ clearly in terms of geographic latitude (data not shown). Only the molar ratios of fucoxanthin+Chl *c* to Chl *a* (Fuc+Chl *c* Chl a^{-1}) were significantly higher at lower latitudes (BSA: 0.39±0.01 and PBL: 0.43±0.01) compared to higher latitudes (TEA: 0.35±0.01, PTAL: 0.36±0.01 and PCH: 0.36±0; p<0.001). However, there were significant differences between the species for the majority of the pigments. The contents of Chl *a* and *c*, Fuc and Fuc+Chl *c* Chl a^{-1} were higher in *L. berteroana* than in *L. spicata*. These species-specific differences could be observed along the full latitudinal range (Chl *a*: p=0.004, Chl *c*: p=0.001, Fuc: p<0.001 and Fuc+Chl *c* Chl a^{-1}: p=0.011; Table 3.3) as well as within the overlapping zone, with the exception of Fuc+Chl *c* Chl a^{-1} (Chl *a*: p=0.003, Chl *c*: p=0.005 and Fuc: p=0.002; Table 3.3). No significant differences between the two species were observed with respect to β-carotene contents (Table 3.3). The pool size of the xanthophyll cycle pigments (VAZ) was larger in *L. berteroana* than in *L. spicata* both along the full latitudinal range (p=0.001; Figure 3.3a) and within the overlapping zone (*L. berteroana*: 0.18±0.01 and *L. spicata*: 0.14±0.01, p=0.001). The DPS, in contrast, showed significant differences with respect to geographic latitude, with highest values in individuals from PBL and lowest in those from TEA (p<0.001; Figure 3.3b).

Table 3.3: *Lessonia berteroana* and *Lessonia spicata*: pigment concentrations (µg mg^{-1} dw) and molar ratio of Fuc+Chl *c* to Chl *a* (Fuc+Chl *c* Chl a^{-1}) of algal samples collected during January to February 2012.

Pigment	Species (full latitudinal range)		Species (overlapping zone)	
	L. berteroana	***L. spicata***	***L. berteroana***	***L. spicata***
Chl *a**	1.57±0.07	1.29±0.06	1.54±0.07	1.20±0.06
Chl *c**	0.11±0.01	0.09±0.00	0.11±0.00	0.09±0.00
Fuc*	0.49±0.02	0.38±0.02	0.69±0.04	0.36±0.02
β-caro	0.08±0.00	0.08±0.00	0.08±0.01	0.08±0.00
Fuc+Chl *c* Chl a^{-1}**	0.38±0.01	0.37±0.00	0.37±0.00	0.37±0.00

Along the full latitudinal range (27°39´S-32°19´S), data for each species are given as pooled values from all four sampling sites (BSA, PBL, APO and TEA for *L. berteroana* and ACE, ERM, PTAL and PCH for *L. spicata*). Within the overlapping zone (29°04´S-29°49´S), data for each species are given as pooled values from the two sampling sites (APO and TEA for *L. berteroana* and ACE and ERM for *L. spicata*). See Table 3.1 for site name abbreviations. Table shows means±SEM (n=20 for full latitudinal range and n=10 for overlapping zone). Asterisks indicate significant differences between species (*in full latitudinal range and overlapping zone, **only in full latitudinal range; independent Student's t test, p<0.05). β-caro, β-carotene; Fuc, fucoxanthin.

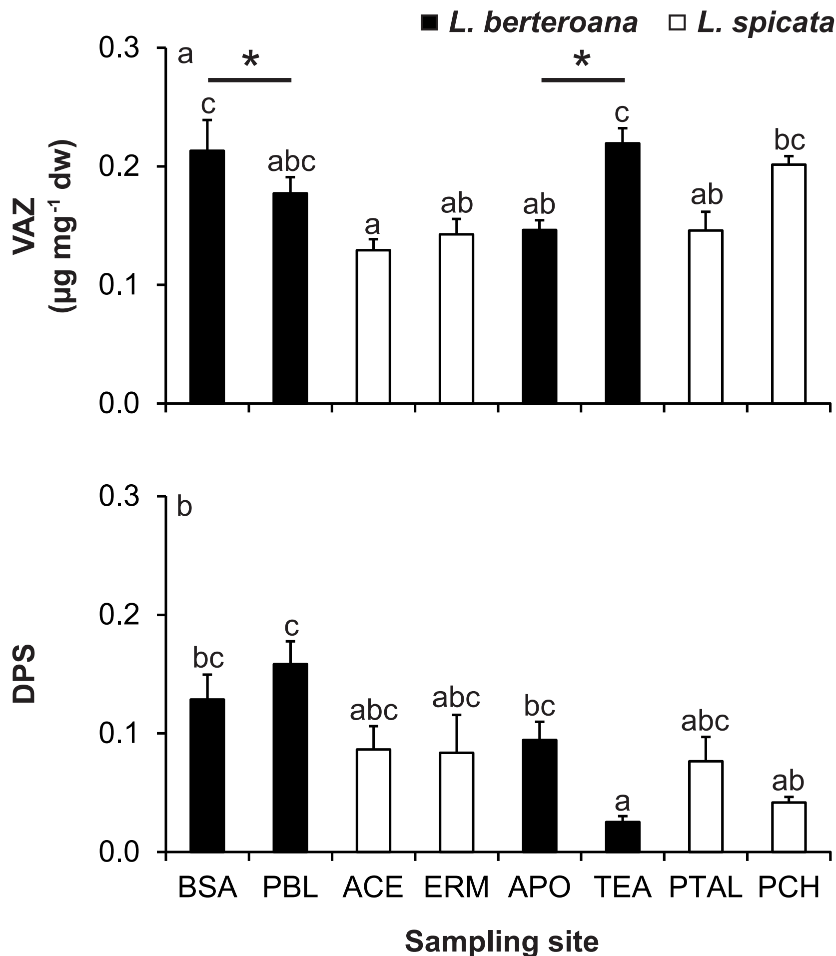

Figure 3.3: *Lessonia berteroana* and *Lessonia spicata*: **(a)** xanthophyll cycle pigment pools (VAZ, µg mg^{-1} dw) and **(b)** de-epoxidation states of xanthophyll cycle (DPS) of algal samples collected at eight different sampling sites during January to February 2012. See Table 3.1 for site name abbreviations. Bars are means±SEM (n=5). Different letters (a, b and c) indicate differences among sampling sites that are significant at p=0.05 (one-way ANOVA followed by a post-hoc Tukey's HSD test). For Student's t test comparisons, individual values of all four sampling sites from *L. berteroana* (BSA, PBL, APO and TEA) were pooled and tested against the corresponding pooled values from *L. spicata* (ACE, ERM, PTAL and PCH). Asterisks indicate species-specific differences between these pooled data that are significant at p=0.05.

Maximum quantum yields of algal samples were within the typical range reported for non-stressed brown algae (data not shown; Büchel and Wilhelm 1993). As for the photosynthetic pigments, the parameters of variable Chl *a* fluorescence of PSII exhibited no clear trends with latitude (data not shown). However, ETR_{max} and E_k showed significant species-specific differences along the full latitudinal range, with 30% and 20% higher values in *L. berteroana* than in *L. spicata*, respectively (ETR_{max}: p=0.005, E_k *L. berteroana*: 480±21 µmol photons m^{-2} s^{-1} and *L. spicata*: 396±21 µmol photons m^{-2} s^{-1}; p=0.008; Figure 3.4). The same pattern was observed within the overlapping zone (ETR_{max}: p<0.001, E_k *L. berteroana*: 546±18 µmol photons m^{-2} s^{-1} and *L. spicata*: 457±17 µmol photons m^{-2} s^{-1}; p=0.001; data not shown). In contrast, α did not differ significantly between the species

(*L. berteroana*: 0.273±0.006 µmol e^- m^{-2} s^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$ and *L. spicata*: 0.256±0.007 µmol e^- m^{-2} s^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$).

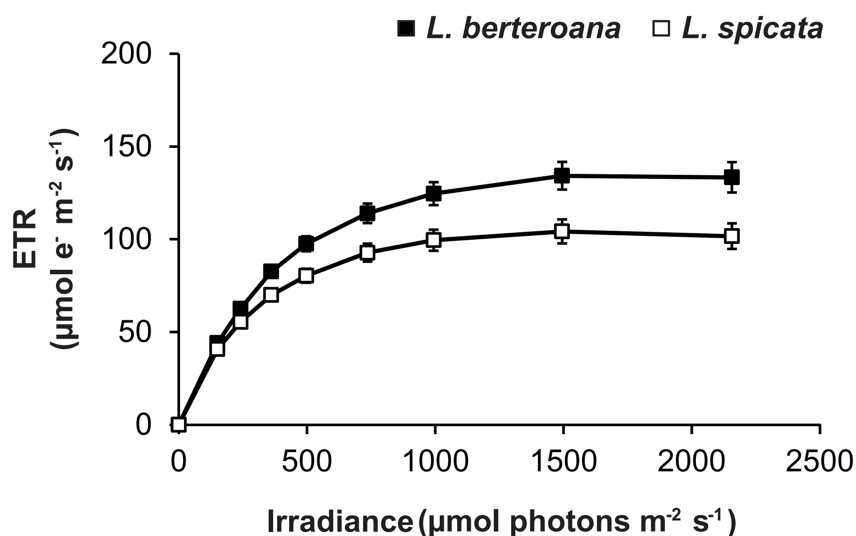

Figure 3.4: *Lessonia berteroana* and *Lessonia spicata*: photosynthesis versus irradiance curves (P-E curves) of algal samples collected during January to February 2012. Curves are based on estimations of electron transport rates (ETR, µmol e^- m^{-2} s^{-1}). For each species, data are given as pooled values from all four sampling sites (BSA, PBL, APO and TEA for *L. berteroana* and ACE, ERM, PTAL and PCH for *L. spicata*; site name abbreviations as in Table 3.1). Data points are means±SEM (n=20). Species-specific differences in maximum electron transport rates (ETR_{max}) and saturating irradiances (E_k; defined by P-E curve fitting after Jassby and Platt (1976)), were found that are significant at p=0.05.

DISCUSSION

Overall, our results reveal that the two cryptic species within the *Lessonia nigrescens* complex exhibit differences in many of the ecophysiological characteristics (phlorotannins, pigments, Chl *a* fluorescence of PSII) tested. These differences were not only found along the full latitudinal range (approximately 520 km of coastline), but also within the narrow overlapping zone (approximately 80 km of coastline), in which populations of *Lessonia berteroana* and *Lessonia spicata* grow in close proximity. Therefore, we propose that the observed ecophysiological differences might be considered as genetically determined species-specific differences, which are not masked by responses to the respective environmental settings along the latitudinal gradient.

The Chilean coast is affected by the Humboldt Current System, and changes in several abiotic parameters followed a latitudinal gradient (Thiel et al. 2007). Generally, solar irradiance and sea surface temperature (SST) decrease with increasing latitude (Broitman et al. 2001, Hernández et al. 2012). However, temperature conditions along the Chilean coast can be highly heterogeneous due to persistent upwelling of cold nutrient-rich subsurface waters at certain locations. Further, the intensity of air exposure during a tidal

cycle decreases towards higher latitudes, as a result of the decline in SST and rise in relative humidity (Thiel et al. 2007, López-Cristoffanini et al. 2013). During these periods of air exposure, intertidal kelps experience numerous stress factors, like high levels of photosynthetically active radiation (PAR), UV, temperature and salinity as well as desiccation and nutrient limitation (Davison and Pearson 1996). Thus, due to their contrasting latitudinal distribution along the Chilean coast (Tellier et al. 2009), *L. berteroana* and *L. spicata* experience differences in environmental conditions. Overall, *L. berteroana* is exposed to higher solar irradiance and SST as well as longer air exposure during a tidal cycle compared to *L. spicata*. Mean annual as well as monthly maximum and minimum seawater temperatures can differ strongly between extreme localities along the distributional range of the *L. nigrescens* species complex (Martínez 1999, Oppliger et al. 2011). Of course, variations in environmental conditions are more pronounced along the total distributional range of the two cryptic species as compared to the much smaller full latitudinal range (27°39´S to 32°19´S) tested in our study. Nonetheless, it was previously shown that differences in abiotic forcings were also detectable within comparable latitudinal gradients along the Chilean coast (Broitman et al. 2001, Oppliger et al. 2011, 2012, Tellier et al. 2011b). For example, Tellier et al. (2011b) found a difference of 5°C between the weekly mean SST (1982-2008) at 25°S and 35°S. López-Cristoffanini et al. (2013) reported that the weekly mean SST ranged from 14 to 20°C at 26°S (Pan de Azúcar) and from 13 to 18°C at 29°S (ERM and APO) as well as at 33°S (Las Cruces). Furthermore, due to the local upwelling region of Punta Lengua de Vaca (approximately 30/31°S), the *Lessonia* populations at the two southernmost locations (PTAL, PCH) of the full latitudinal range are thought to be continuously exposed to colder and more nutrient-rich waters, whereas populations at the more northern locations (BSA to TEA) may be temporarily exposed to warmer waters with less nutrients. However, local hydrodynamic features may further modulate temperature conditions on a smaller scale (Thiel et al. 2007, Tapia et al. 2014). The global horizontal irradiance (2003-2012) tended to decrease from BSA to PCH by about 5% (Explorador de energía solar, http://walker.dgf.uchile.cl/Explorador/Solar2/).

The species-specific differences in ecophysiological characteristics may allow *L. berteroana* and *L. spicata* to survive and grow at the prevailing abiotic conditions at their respective growth sites. Both cryptic species followed the well-known pattern of photosynthetic acclimation to various solar irradiances along their distributional range (Reiskind et al. 1989, Marquardt et al. 2010), displaying higher values of ETR_{max} and E_k in *L. berteroana* than in *L. spicata*. This species-specific adaptation enables photosynthesis of *L. spicata* to be saturated already at lower irradiances. With respect to photosynthetic pigments, species-specific differences were found for Chl *a* and *c* as well as Fuc. However, *L. berteroana* exhibited higher concentrations of those pigments than *L. spicata,* which is

contradictory to the expected and typical photoacclimatory adjustments with reduced relative amounts of antenna pigments in high light environments (e.g., Wheeler 1980, Smith and Melis 1987). Why both cryptic *Lessonia* species reacted contrarily to this expectation remains unresolved. Further, VAZ displayed species-specific differences, whereas DPS showed differences with respect to geographic latitude. The xanthophyll cycle is known to play a key role in the dissipation of excess light energy via non-photochemical quenching and thus in the protection of the photosynthetic apparatus against photodamage. Thereby, adjustments of the pigment pool size (VAZ) were considered to be long-term responses that mirror the protective activity of the xanthophyll cycle (Pfündel and Bilger 1994). We propose that the enhanced VAZ in *L. berteroana* compared to *L. spicata* forms part of the acclimation response to higher solar irradiances at its growth sites. Colombo-Pallotta et al. (2006) described the same acclimation response with respect to VAZ in highly irradiance-exposed surface blades of the giant kelp *Macrocystis pyrifera*. In contrast, adjustments of the rates of xanthophyll cycle pigment conversion (DPS) were defined as short-term responses (Pfündel and Bilger 1994), potentially caused by prevailing changes in solar irradiance along the latitudinal gradient of the Chilean coast. The DPS, however, showed significant differences with respect to geographic latitude, with highest values in individuals from PBL and lowest in those from TEA ($p<0.001$; Figure 3.3b).

As for the pigments, soluble phlorotannin contents displayed species-specific differences. Phlorotannins are phenolic compounds with several putative secondary functions such as herbivore deterrence, scavenging of reactive oxygen species (ROS) and screening against potentially harmful UV radiation (Koivikko et al. 2005). Since *L. berteroana* experiences higher levels of UV and possibly enhanced ROS formation, both in the water column and in the air during low tide, we suggest that larger amounts of phlorotannins mirror the species' latitudinal spread. Similar results were found by Cruces et al. (2012), who detected enhanced phlorotannin contents in *L. spicata* under high PAR and especially under high UV conditions. Additionally, larger amounts of phlorotannins might allow *L. berteroana* to tolerate the potentially higher grazing pressure, which was found to increase at lower latitudes (Broitman et al. 2001).

Overall, total lipid contents in *L. berteroana* and *L. spicata* were relatively low (approximately 2% of dw). This agrees with a study on five macroalgal species by Herbreteau et al. (1997), who also propose that very low total lipid levels appear to be characteristic for plants living in marine environments. However, Westermeier and Gómez (1996) determined total lipid contents of around 0.4% of dw for fronds of the *L. nigrescens* complex. To our knowledge, fatty acid compositions of the two cryptic species are described for the first time in the present study, but differences in abiotic conditions within the contrasting distribution ranges of the two species were hardly reflected by their fatty acid

compositions. The fatty acid composition is an important determinant of membrane fluidity, which is essential to maintain photosynthetic functions (e.g., re-integration of *de novo* synthesized D1 reaction center proteins; Becker et al. 2010 and references therein). Previous studies have shown that differences in abiotic conditions such as light and temperature may result in changes of the fatty acid composition and metabolism of macroalgae (e.g., Pettitt et al. 1989, Khotimchenko and Yakovleva 2005, Becker et al. 2010). Nonetheless, according to our results, adjustments of fatty acid composition seem to play a negligible role in the adaptive processes in the two cryptic species of the *L. nigrescens* complex.

CONCLUSION

The results of the present study confirm that species differentiation within the *L. nigrescens* complex is also manifested at the biochemical level. Of all parameters tested, phlorotannins, pigments and Chl *a* fluorescence of PSII seem to be the most crucial for species-specific adaptations to the prevailing abiotic conditions at the respective growth sites, whereas differences in total lipids and fatty acid compositions were non-existent between the two species (exception fatty acid 16:0). These new findings with respect to the ecophysiology of *Lessonia berteroana* and *Lessonia spicata* might help to explain their differences in tolerances to temperature and air exposure (Martínez 1999, Oppliger et al. 2011, 2012, López-Cristoffanini et al. 2013) or to predict their responses to abiotic stresses (e.g., rise of SST during El Niño Southern Oscillation events; Martínez et al. 2003). To test whether our findings are also valid for more marginal *Lessonia* populations, future studies should extend the characterization of ecophysiological characteristics to the total distributional range of both cryptic species. Furthermore, gene expression studies are suggested, which will allow in-depth insights into physiological implications of speciation processes along environmental gradients.

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4 PUBLICATION II

SHORT- AND LONG-TERM ACCLIMATION PATTERNS OF THE GIANT KELP *MACROCYSTIS PYRIFERA* (LAMINARIALES; PHAEOPHYCEAE) ALONG A DEPTH GRADIENT

Kristina Koch[1,2], Martin Thiel[3,4,5], Wilhelm Hagen[2,6], Martin Graeve[7], Iván Gómez[8], David Jofre[3], Laurie C. Hofmann[1,2,9], Fadia Tala[3,10] and Kai Bischof[1,2]

[1]Marine Botany, University of Bremen, Bremen, Germany
[2]Bremen Marine Ecology – Center for Research and Education (BreMarE), University of Bremen, Bremen, Germany
[3]Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile
[4]Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, Chile
[5]Nucleus Ecology and Sustainable Management of Oceanic Island (ESMOI), Coquimbo, Chile
[6]Marine Zoology, University of Bremen, Bremen, Germany
[7]Ecological Chemistry, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany
[8]Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile
[9]Max Planck Institute for Marine Microbiology, Bremen, Germany
[10]Centro de Investigación y Desarrollo Tecnológico en Algas (CIDTA), Coquimbo, Chile

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ABSTRACT

The giant kelp, *Macrocystis pyrifera*, is exposed to highly variable irradiance and temperature regimes across its geographic and vertical depth gradients. The objective of this study was to extend our understanding of algal acclimation strategies on different temporal scales to those varying abiotic conditions at various water depths. Different acclimation strategies to various water depths (0.2 and 4 m) between different sampling times (Jan/Feb and Aug/Sept 2012; long-term acclimation) and more rapid adjustments to different depths (0.2, 2 and 4 m; short-term acclimation) during 14 days of transplantation were found. Adjustments of variable Chl *a* fluorescence, pigment composition (Chl *c*, fucoxanthin), and the de-epoxidation state of the xanthophyll cycle pigments were responsible for the development of different physiological states with respect to various solar radiation and temperature climates. Interestingly, the results indicated that phlorotannins are important during long-term acclimation while antioxidants have a crucial role during short-term acclimation. Furthermore, the results suggested that modifications in total lipids and fatty acid compositions apparently also might play a role in depth acclimation. In Aug/Sept (austral winter), *M. pyrifera* responded to the

transplantation from 4 m to 0.2 m depth with a rise in the degree of saturation and a switch from shorter- to longer-chain fatty acids. These changes seem to be essential for the readjustment of thylakoid membranes and might, thus, facilitate efficient photosynthesis under changing irradiances and temperatures. Further experiments are needed to disentangle the relative contribution of solar radiation, temperature and also other abiotic parameters in the observed physiological changes.

KEYWORDS

Acclimation, antioxidants, Chile, fatty acid composition, PAR, phlorotannins, temperature, total lipids

ABBREVIATIONS

α, initial linear slope of P-E curve (photosynthetic efficiency); ANOVA, analysis of variance; Aug/Sept, August/September; β-caro, β-carotene; Di, incubation depth; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DPS, de-epoxidation state of the xanthophyll cycle pigments; Ds, sampling depth; dw, dry weight; E_k, saturating irradiance; ETR, electron transport rate; ETR_{max}, maximum electron transport rate (photosynthetic capacity); FA, fatty acid; FAME, fatty acid methyl ester derivate; Fuc, fucoxanthin; F_v/F_m, maximum quantum yield; HSD, honest significant difference; Jan/Feb, January/February; K_d, vertical attenuation coefficient of downward irradiance; Max, maximum; Min, minimum; n.d., not detected; n.m., not measured; NPQ, non-photochemical quenching of Chl a fluorescence; PAM, pulse amplitude modulation; PCO, principal coordinate analysis; P-E curve, photosynthesis versus irradiance curve; PERMANOVA, permutational multivariate analysis of variance; PERMDISP, permutational analysis of multivariate dispersion; ratio sat/unsat FA, saturated/unsaturated fatty acid ratio; ROS, reactive oxygen species; SD, standard deviation; SEM, standard error of the mean; SIMPER, similarity percentage analysis; TE, Trolox equivalent; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Ts, sampling time; UCN, Universidad Católica del Norte; VAZ, xanthophyll cycle pigment pool; ww, wet weight

INTRODUCTION

The giant kelp, *Macrocystis pyrifera* (L.) C. Agardh, reaches sizes up to 60 m, and hence, a single sporophyte may span the entire water column from the bottom to the sea surface (North 1994). The kelp is attached to the substratum by a hapterous holdfast and its numerous fronds are maintained vertically in the water column by gas-filled pneumatocysts that may reach the sea surface (North 1994), where they form a dense floating canopy (Gerard 1984, Dean 1985). *Macrocystis pyrifera* is an ecologically important species

throughout its distribution range along the west coast of North America and cold-temperate waters of the southern hemisphere with growing economic importance in South America and southern California, USA (Buschmann et al. 2014a, Westermeier et al. 2014). The species exhibits high primary production rates (Towle and Pearse 1973, North 1994) and provides three-dimensional habitats, food sources and nursery grounds for associated invertebrates and fishes (Graham et al. 2007, Pérez-Matus et al. 2007, Villegas et al. 2008). Additionally, sporophytes may become detached from the substratum and may act as long-distance dispersal vectors during their extensive free-floating periods (approximately 1000 km in 100 days; Macaya et al. 2005, Thiel and Haye 2006, Rothäusler et al. 2012).

Given its ability to grow along extensive latitudinal and vertical depth gradients and to float at the sea surface, the giant kelp must have developed various acclimation mechanisms to cope with the extreme variability in abiotic conditions. For example, surface waters exhibit distinct temperature differences along the wave-exposed Chilean coast, with summer values of >20°C in the North and <14°C in the South (Rothäusler et al. 2009). In the wave-protected channels and fjords in southern Chile, temperature can even range from 8°C to 15°C (Buschmann et al. 2004). Further, within a kelp forest, pronounced vertical gradients of irradiance, temperature, and nutrients exist (North 1971, Dean 1985). The prevailing sunlight at the surface can be reduced by 63%-78% directly under the canopy (Huovinen and Gómez 2011) and by 99% at 20 m depth (Dean 1985).

Several studies, focusing mainly on photosynthesis and pigments of *M. pyrifera* under different temperature and light conditions, revealed that algal acclimation patterns depend on the position of the photosynthetic tissue in the water column. Gerard (1986) found that surface blades of the giant kelp from southern California exhibit no short-term photoinhibition under high light conditions, whereas the photosynthetic rates of deeper blades are strongly depressed during exposure to the same irradiance. Also, photoprotection (e.g., accumulation of xanthophyll cycle pigments and increase in non-photochemical quenching (NPQ) rates of Chl *a* fluorescence) is favored in surface blades (Colombo-Pallotta et al. 2006, García-Mendoza and Colombo-Pallotta 2007), whereas light-harvesting is enhanced in deeper blades (Wheeler 1980, Smith and Melis 1987).

Adjustments of phlorotannin and antioxidant levels are further known to contribute to the acclimation potential of Phaeophyceae to different abiotic conditions. Phlorotannins are suggested to act as sunscreen substances against potentially harmful solar radiation (high PAR and UV) in brown algae (Schoenwaelder and Clayton 1998, Schoenwaelder 2002, Swanson and Fox 2007, Cruces et al. 2012). These authors further detected a correlation between phlorotannins and antioxidants, which function as scavengers of reactive oxygen species (ROS), in *M. pyrifera*. Since ROS can damage proteins and lipids, thylakoid membrane integrity might not be maintained and stress-induced repair processes of

membranes might be slowed down, resulting in severe decreases of photosynthetic capacity (Bischof and Rautenberger 2012). Thus, changes in abiotic conditions may also result in adjustments of the fatty acid (FA) composition (e.g., Becker et al. 2010). Optimum membrane fluidity, which is mainly determined by the FA chain length and degree of FA saturation, is essential for proper cell functioning. It is generally accepted that at low temperatures, membranes exhibit higher amounts of shorter-chain and unsaturated FAs with lower melting points. At high temperatures, in contrast, more longer-chain and saturated FAs with higher melting points are incorporated into membranes (Buchanan et al. 2000). Some previous studies pointed out that changes in environmental parameters can cause alterations of the FA profiles of marine macrophytes (e.g., Pettitt et al. 1989, Pettitt and Harwood 1989, Schmid et al. 2014), with habitat conditions rather than the geographical location determining differences in macroalgal FA compositions (Khotimchenko et al. 2002). There is also evidence for seasonal variability in lipid class composition, which was shown for *Ulva lobata* (Chlorophyta), *Egregia menziesii* (Phaeophyceae) and *Chondracanthus canaliculatus* (Rhodophyta) (Nelson et al. 2002).

Based on the above considerations, the objective of this study was to extend our understanding of long- and short-term acclimation strategies of *M. pyrifera* to varying environmental conditions at different water depths. Given the multitude of abiotic factors, shaping the algal environments at various water depths, we concentrated specifically on light and temperature climates in this study. We further focused on kelp sporophytes from intertidal and shallow subtidal habitats because they experience strong fluctuations in abiotic factors. Furthermore, the kelps from those habitats are exposed to wave action and they may consequently experience a high detachment risk. Subsequent floating to the sea surface is followed by abrupt changes in environmental conditions. Since kelp responses to changing abiotic regimes are known to differ between seasons (e.g., Tala et al. 2013), the study was conducted once in January/February and again in August/September (hereafter Jan/Feb and Aug/Sept) of 2012. We refer to long-term responses as consistent acclimation strategies between different sampling times (Jan/Feb and Aug/Sept), whereas short-term responses were measured after transplantation of kelp fronds to different water depths for 14 days. Kelp responses were determined by measurements of variable Chl *a* fluorescence of PSII and various biochemical parameters (pigment composition, phlorotannin content, antioxidant activity, total lipid content). Another major focus of this study, which had been hardly addressed, was to examine the adjustments in FA composition and their importance in acclimation processes of *M. pyrifera*. We hypothesized that stress tolerance mechanisms, particularly in Jan/Feb samples from surface waters (i.e., during exposure to highest solar radiation and temperature), should operate in a way that phlorotannin and antioxidant levels

increase along with membrane FAs with high melting points and that these parameters would further differ with depth.

MATERIALS AND METHODS

Algal material and sampling site

Approximately 1.5 m long apical fronds containing stipe, apical meristem and blades were collected at low tide from two *M. pyrifera* kelp forests at the coast of northern-central Chile. Kelp fronds were sampled at approximately 0.2 m water depth in Punta de Talca (30°50`S, 71°41`W) and at approximately 4 m water depth in Playa Blanca (28°11`S, 71°09`W). Since along the coast of northern-central Chile almost no *Macrocystis* populations exist in which sporophytes grow at both water depths, kelp fronds had to be collected at two different locations. Thus, results of this study should be interpreted with the understanding that depth effects are potentially influenced by spatial differences in location of source plants. However, we consider spatial effects to be minimal since: (i) the same haplotype of *M. pyrifera* occurs at both locations, suggesting that there are no genetic differences between the kelp individuals from the two sites (Macaya and Zuccarello 2010) and (ii) although climatic as well as oceanographic conditions can vary locally at the two distinct sites (in the past and/or present), overall, abiotic parameters are comparable within coastal regions of northern-central Chile (Hormazabal and Shaffer 2002, Garreaud et al. 2011, Tapia et al. 2014).

At each location, 20 apical fronds were taken from independent sporophytes in Jan/Feb and Aug/Sept 2012. Apical kelp fronds were kept in darkness and immediately transported in coolers with seawater at ambient water temperature to the marine laboratory at Universidad Católica del Norte (UCN) in Coquimbo, Chile (29°57`S, 71°20`W), where they were stored overnight in large outdoor flow-through seawater tanks (approximately 2000 l).

Measurement of abiotic conditions at various water depths

Since in this study apical kelp fronds were incubated at different water depths in the Bahía La Herradura, Coquimbo, Chile, a bay located in the vicinity of the marine laboratory at the UCN, prevailing irradiance of PAR (400-700 nm) and water temperature were monitored throughout the course of the experiment (Table 4.1). Underwater PAR was measured around noon (12:00-14:00 h, local time) every 1-3 days with a LI-192 cosine corrected underwater quantum sensor connected to a LI-1400 data logger (LiCor, Lincoln, NE, USA) at the three water depths. These measurements were conducted at three different positions along the line of buoys (for details see below) and respective mean values were calculated. In addition, the vertical attenuation coefficient of downward irradiance (K_d) was calculated after Kirk (1994). Water temperature was determined every 3 min with a Hobo® TidbiT v2 water

temperature logger (Onset Computer Cooperation, Bourne, MA, USA) at 0.2 m and 6 m depth. Due to logistical constraints, temperature measurements at 2 and 4 m water depth were not possible. PAR and water temperature data were expressed as total mean values during the 14-day experiment.

Experimental test of short- and long-term depth acclimation

We conducted a 14-day transplantation experiment in the semi-enclosed Bahía La Herradura, Coquimbo, Chile to investigate short-term acclimation responses of *M. pyrifera* to varying light and temperature climates at different water depths. The experiment was performed once in Jan/Feb and repeated in Aug/Sept. At the start of the experiment (day 0), five initial apical kelp fronds (hereafter initial fronds) were selected for the determination of the physiological status of *M. pyrifera* in the field, while the remaining 15 apical fronds were prepared for the transplantation experiment. For this, apical kelp fronds were shortened to a length of approximately 1 m (including the stipe with the apical meristem and seven subsequent free intact blades; hereafter experimental fronds) and tethered at three different depths (0.2, 2, and 4 m) along vertical lines. Five vertical lines were used for experimental fronds from 0.2 m sampling depth (Punta de Talca) and five vertical lines were used for experimental fronds from 4 m sampling depth (Playa Blanca). Each of these vertical lines was equipped with one experimental frond at 0.2 m, one at 2 m and one at 4 m incubation depth. The vertical lines were weighed down by a stone and tied to a line of buoys in the bay with 1-2 m distance from each other. The assignation of experimental fronds to depth treatments and the positioning of the vertical lines along the line of buoys was randomized. Wave action led to losses of experimental fronds, so that in exceptional cases fewer than five fronds per water depth (0.2, 2, and 4 m) were left at the end of the experiment (for details see Tables 4.2 and 4.3, Tables S4.1 and S4.2 in the Supporting Information and Figure 4.1).

Table 4.1: Average, minimum and maximum **(a)** PAR (µmol photons m^{-2} s^{-1}); **(b)** vertical attenuation coefficients of downward irradiance (K_d; m^{-1}); and **(c)** seawater temperatures (°C), measured during the entire experimental duration in January/February (Jan/Feb) and August/September (Aug/Sept) 2012 in the Bahía La Herradura, Coquimbo, Chile. K_d was calculated for the entire experimental depth gradient (0.2-4 m).

	Jan/Feb 2012				Aug/Sept 2012			
	0.2 m	**2 m**	**4 m**	**6 m**	**0.2 m**	**2 m**	**4 m**	**6 m**
(a) PAR								
Mean±SD	1777±351	756±174	389±141	n.m.	818±454	310±235	173±144	n.m.
Min	1050	400	200	n.m.	153	27	4	n.m.
Max	2200	1100	750	n.m.	1720	964	671	n.m.
(b) K_d								
Mean±SD		0.41±0.07				0.46±0.12		
Min		0.26				0.24		
Max		0.59				0.94		
(c) Temperature								
Mean±SD	18.0±0.9	n.m.	n.m.	16.5±1.0	13.9±0.4	n.m.	n.m.	13.6±0.4
Min	15.7	n.m.	n.m.	13.4	13.1	n.m.	n.m.	13.0
Max	20.3	n.m.	n.m.	18.8	15.1	n.m.	n.m.	14.6

Max, maximum; Min, minimum; n.m., not measured; SD, standard deviation.

Table 4.2: Characteristics of variable Chl *a* fluorescence of PSII of initial fronds of *Macrocystis pyrifera* (Day 0) from two different sampling depths (0.2 and 4 m) and experimental fronds of *M. pyrifera* (Day 14), transplanted along the depth gradient (0.2 and 4 m) during January/February (Jan/Feb) and August/September (Aug/Sept) 2012.

Parameter of Chl *a* fluorescence	Sampling depth (m)	Incubation depth (m)	Jan/Feb 2012 Day 0	Jan/Feb 2012 Day 14 (% of initial)	Aug/Sept 2012 Day 0	Aug/Sept 2012 Day 14 (% of initial)
F_v/F_m	0.2	0.2	0.660±0.007	90±4	0.728±0.009	96±3
	0.2	4		115±2		99±2
	4	0.2		100[a]		96±1
	4	4	0.620±0.020	107±3	0.681±0.004	106±3[b]
ETR_{max}	0.2	0.2	61.88±3.63	187±12	31.75±3.64	98±6
	0.2	4		219±10		108±13
	4	0.2		555[a]		161±18
	4	4	28.55±2.00	436±16	19.23±1.64	124±22[b]
E_k	0.2	0.2	286.98±19.06	251±17	121.60±18.12	94±7
	0.2	4		154±9		107±16
	4	0.2		763[a]		137±7
	4	4	120.30±17.49	468±68	87.69±10.32	96±18[b]
α	0.2	0.2	0.217±0.009	76±9	0.266±0.009	103±3
	0.2	4		143±5		96±18
	4	0.2		81[a]		100±9
	4	4	0.248±0.024	94±9	0.223±0.013	114±8[b]

Additionally, experimental fronds were also transplanted to 2 m water depth. Those data are given in Table S4.2 in the Supporting Information. Different PAM devices were used for the measurements in Jan/Feb and Aug/Sept. To get an impression of the status of variable Chl *a* fluorescence in the field, data for day 0 are given as absolute values (F_v/F_m in relative units, ETR_{max} in µmol $e^- m^{-2} s^{-1}$, E_k in µmol photons $m^{-2} s^{-1}$ and α in µmol $e^- m^{-2} s^{-1}$ (µmol photons $m^{-2} s^{-1})^{-1}$). For a better comparability between both sampling times, day 14 values are expressed as % of initial. Table shows means±SEM (n=5 for day 0 and 14, with exceptions of [a]n=2 and [b]n=4).

α, initial linear slope of photosynthesis versus irradiance curve; E_k, saturating irradiance; ETR_{max}, maximum electron transport rate; F_v/F_m, maximum quantum yield; SEM, standard error of the mean.

Table 4.3: Pigment concentrations (µg mg^{-1} dw) and DPS of initial fronds of *Macrocystis pyrifera* (Day 0) from two different sampling depths (0.2 and 4 m) and experimental fronds of *M. pyrifera* (Day 14), transplanted along the depth gradient (0.2 and 4 m) during January/February (Jan/Feb) and August/September (Aug/Sept) 2012.

Pigment	Sampling depth (m)	Incubation depth (m)	Jan/Feb 2012		Aug/Sept 2012	
			Day 0	Day 14	Day 0	Day 14
Chl *a*	0.2	0.2	1.88±0.25	0.82±0.15	2.24±0.36	1.35±0.22
	0.2	4		1.96±0.13		2.32±0.18
	4	0.2		1.05[a]		1.29±0.06
	4	4	2.39±0.29	2.07±0.21	2.27±0.22	2.06±0.42[b]
Chl *c*	0.2	0.2	0.15±0.02	0.05±0.02	0.20±0.04	0.11±0.02
	0.2	4		0.16±0.01		0.26±0.03
	4	0.2		0.11[a]		0.11±0.02
	4	4	0.30±0.03	0.21±0.02	0.24±0.03	0.25±0.06[b]
Fuc	0.2	0.2	0.62±0.09	0.29±0.05	0.79±0.15	0.46±0.08
	0.2	4		0.68±0.04		0.93±0.09
	4	0.2		0.42[a]		0.40±0.06
	4	4	1.11±0.08	0.80±0.09	1.00±0.12	0.87±0.17[b]
β-caro	0.2	0.2	0.08±0.01	0.06±0.01	0.12±0.01	0.10±0.02
	0.2	4		0.10±0.01		0.11±0
	4	0.2		0.07[a]		0.06±0.01
	4	4	0.07±0.01	0.08±0.01	0.10±0	0.09±0.01[b]
VAZ	0.2	0.2	0.18±0.02	0.11±0.02	0.21±0.03	0.16±0.02
	0.2	4		0.17±0.01		0.20±0.02
	4	0.2		0.11[a]		0.10±0
	4	4	0.15±0.03	0.13±0.01	0.21±0.01	0.17±0.04[b]
DPS	0.2	0.2	0.054±0.018	0.141±0.021	0.074±0.019	0.115±0.024
	0.2	4		0.039±0.008		0.037±0.014
	4	0.2		0.180[a]		0.073±0.023
	4	4	0.085±0.012	0.071±0.019	0.017±0.001	0.047±0.018[b]

Additionally, experimental fronds were also transplanted to 2 m water depth. Those data are given in Table S4.1 in the Supporting Information. Table shows means±SEM (n=5 for day 0 and 14, with exceptions of [a]n=2 and [b]n=4).

β-caro, β-carotene; DPS, de-epoxidation state of xanthophyll cycle pigments; Fuc, fucoxanthin; SEM, standard error of the mean; VAZ, xanthophyll cycle pigment pool.

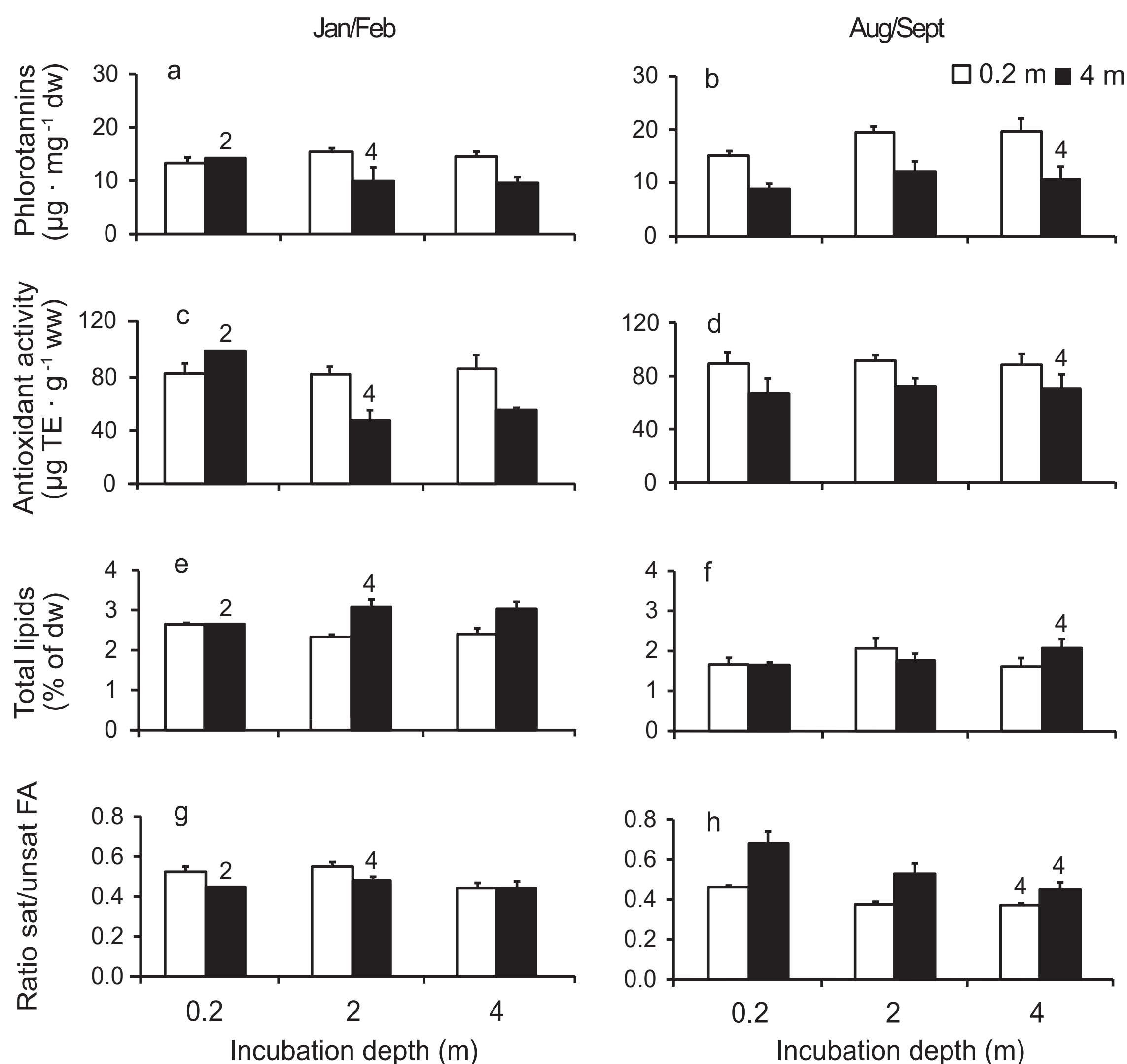

Figure 4.1: (a, b) Soluble phlorotannin contents (µg mg^{-1} dw), **(c, d)** antioxidant activities (µg TE g^{-1} ww), **(e, f)** total lipid contents (% of dw) and **(g, h)** saturated/unsaturated fatty acid ratios (ratio sat/unsat FA) of experimental fronds of *Macrocystis pyrifera*, collected at two different sampling depths (0.2 and 4 m) and transplanted for 14 days along the depth gradient (0.2, 2, and 4 m) during January/February (Jan/Feb) and August/September (Aug/Sept) 2012. Figure shows means±SEM (n=5, exceptions are represented by numbers above bars).

Measurement of algal responses to short- and long-term depth exposure

At the start of the transplantation experiment (day 0), all response variables (see below) were measured from five initial fronds to determine long-term acclimation responses of *M. pyrifera* to both sampling times and depths. To test further for the transplantation effects on each physiological variable of experimental fronds, their short-term responses were monitored on day 14 of the experiment. Thereby, due to the limitations of the chosen experimental set-up, algal fronds, sampled at 0.2 or 4 m water depth and incubated at the same depth during the transplantation experiment, respectively, had to serve as controls. Each sampling day (day 0 and 14) started at 09:00 (local time), when vegetative blades of each initial and experimental fronds were sampled and gently cleaned from epibionts. Since individual blades of apical

kelp fronds differ in their age and developmental history, we pooled blades for measurements of the different response variables in order to obtain representative replicate samples from each apical frond. For each apical frond, a total of 16 disks (1.5 cm diameter) were cut from three vegetative blades with a cork borer. After pooling these 16 disks from each replicate frond in each treatment group, four blade disks were selected at random and pooled as one replicate sample for each biochemical analysis. Measurements of Chl *a* fluorescence were carried out immediately, whereas the samples for the other physiological analyses were shock-frozen in liquid nitrogen and stored at -80°C until further processing.

Chl a fluorescence

In vivo Chl *a* fluorescence of PSII was measured with a pulse amplitude-modulated fluorometer (Diving-PAM and PAM 2500; Walz, Effeltrich, Germany in Jan/Feb and in Aug/Sept, respectively). For logistical reasons, different fluorometers had to be used during Jan/Feb and Aug/Sept. All measurements were conducted from 09:00 to 12:00 h (local time) to reduce any variation in the determined parameters due to diurnal periodicity. The maximum quantum yield (F_v/F_m) was measured in dark adapted (5 min) blade disks. Electron transport rates (ETR) were estimated from rapid photosynthesis versus irradiance curves (P-E curves). The blade disks were irradiated with a series of stepwise increasing actinic irradiances (approximately 150-2150 µmol photons m^{-2} s^{-1} in Jan/Feb and approximately 10-1700 µmol photons m^{-2} s^{-1} in Aug/Sept) at 30-s intervals, provided by the respective actinic light source of the PAM devices (Schreiber et al. 1994). Subsequently, the photosynthetic capacity (ETR_{max}, maximum electron transport rate), the photosynthetic efficiency (α, initial linear slope) and the saturating irradiance (E_k) were defined by P-E curve fitting after Jassby and Platt (1976). Since two PAM devices with different actinic light sources were used for Chl *a* fluorescence measurements during Jan/Feb and Aug/Sept, the means of the initial values (day 0) were normalized to 100%. This allows a better comparability between samples from both sampling times. We are aware that reliability of P-E parameters derived from a Diving-PAM is hampered by potential shifts in the emission spectra during recording. However, those limitations do not affect our general results, as we only accounted for the relative change in parameters.

Pigments

Pigment determination was performed by reversed phase HPLC. The four pooled blade disks from each replicate sample were lyophilized together for 24 h and pulverized at 4 m s^{-1} for 20 s in a high-speed benchtop homogenizer (FastPrep®-24; MP Biomedicals, Solon, OH, USA). Pigments from 20 to 50 mg dry weight of blade disks were extracted in 1 mL of ice-cold 90% acetone for 24 h at -20°C in the dark. After centrifugation (5 min, 4°C, 13000 *g*) and

filtration through a 45 µm nylon syringe filter (Nalgene®, Nalge Nunc International, Rochester, NY, USA), HPLC analysis was performed on a LaChromElite® system equipped with a chilled autosampler L-2200 and a DAD detector L-2450 (VWR-Hitachi International GmbH, Darmstadt, Germany). A Spherisorb® ODS-2 column (25 cm x 4.6 mm, 5 µm particle size; Waters, Milford, MA, USA) with a LiChrospher® 100-RP-18 guard cartridge was used for the separation of pigments, applying a gradient according to Wright et al. (1991). Peaks were detected at 440 nm and identified as well as quantified by co-chromatography with standards for Chl *a* and *c*, fucoxanthin (Fuc), β-carotene (β-caro), violaxanthin, antheraxanthin, and zeaxanthin (DHI Lab Products, Hørsholm, Denmark) using the software EZChrom Elite ver. 3.1.3. (Agilent Technologies, Santa Clara, CA, USA). Pigment contents were expressed as micrograms per milligram dry weight. The de-epoxidation state (DPS) of the xanthophyll cycle pigments was calculated as described in Colombo-Pallotta et al. (2006).

Phlorotannins

The total soluble phlorotannin content was determined using the Folin-Ciocalteu method described in Cruces et al. (2012). Purified phloroglucinol (Sigma-Aldrich, Seelze, Germany) was used as a standard. The four pooled blade disks from each replicate sample were lyophilized as described above. Soluble phlorotannins from 10 mg dry weight of blade disks were extracted in 1 mL of 70% acetone for 24 h at 4°C under shaking. After centrifugation (10 min, 4°C, 2500 *g*), 50 µL of the supernatant was mixed with 250 µL of deionized water (dH_2O), 200 µL of 20% sodium carbonate ($NaCO_3$) and 100 µL of 2N Folin-Ciocalteu reagent (Sigma-Aldrich, Seelze, Germany). After 45 min of incubation at room temperature in the dark and centrifugation (3 min, room temperature, 2000 *g*), the absorbance was read at 730 nm using a microplate reader (FLUOstar OPTIMA; BMG Labtech GmbH, Ortenberg, Germany). Soluble phlorotannin contents were estimated from triplicate subsamples, from which one mean was calculated, and defined as micrograms per milligram dry weight.

Antioxidant activity

The antioxidant activity was measured by the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich, Seelze, Germany) scavenging method according to Brand-Williams et al. (1995) as modified by Cruces et al. (2012). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich, Seelze, Germany) was used as a standard. A 150 µM DPPH* stock solution was prepared in 80% ethanol. The four pooled blade disks from each replicate sample were ground together in a mortar with liquid nitrogen. Antioxidants from 50 mg wet weight of blade disks were extracted in 1500 µL of 70% acetone for 24 h at 4°C while shaking in the dark. Afterwards, 22 µL of the supernatant and 200 µL of the DPPH* stock solution were directly mixed in a 96-well microplate. After

15 min, the absorbance was measured at 520 nm using a Multiskan Spectrum microplate photometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The antioxidant activity was estimated from triplicate subsamples, from which one mean was calculated, and expressed as microgram Trolox equivalent (TE) per gram wet weight.

Total lipid content and fatty acid composition

The four pooled blade disks from each replicate sample were lyophilized together for 48 h and pulverized at 1500 rpm for 1 min with liquid nitrogen in a micro-dismembrator (Typ U, B. Braun Biotech International GmbH, Melsungen, Germany). Total lipids were extracted in dichloromethane:methanol (2:1 per volume) following the methods described by Folch et al. (1957) and Bligh and Dyer (1959). Extracts were pestled and ultrasonicated and total lipid contents were determined gravimetrically after Hagen (2000). For the analysis of FA composition, aliquots of the algal extracts were taken. FAs were converted to their methyl ester derivates (FAMEs) by transesterification with methanol containing 3% concentrated sulphuric acid for 4 h at 80°C. After extracting the FAMEs three times with hexane, their composition was analyzed using a HP 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a DB-FFAP column (60 m length, 0.25 mm inner diameter, 0.25 µm film thickness; Agilent Technologies) operated with temperature programming according to the method of Kattner and Fricke (1986). FAMEs were identified by comparing their retention times with those derived from standards of known composition. Individual FAs are presented as mass percentage of total FAs. Based on the individual FA composition, they are grouped according to their degree of saturation and chain length.

Statistical analysis

To test for differences in temperature and PAR related to the factors sampling time (two levels: Jan/Feb and Aug/Sept) and incubation depth (two levels for temperature: 0.2 and 6 m, three levels for PAR: 0.2, 2 and 4 m), two-factorial analyses of variance (two-way ANOVA) were carried out. When the ANOVA revealed significant differences in PAR, a post-hoc Tukey's honest significant difference (HSD) test was applied. To evaluate differences in K_d between the two different sampling times (Jan/Feb and Aug/Sept), an independent Student's t test was conducted. Further, all abiotic data were tested for normality and homogeneity of variances, using Kolmogorov-Smirnov's test and Levene's test, respectively. The software IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA) was used for statistical analyses of abiotic parameters. Critical significance levels of 5% were used.

Physiological data were analyzed by permutational multivariate analyses of variance (PERMANOVA), based on Euclidean distances. This statistical method was chosen because it allows the testing of complex multivariate experimental designs while maintaining

robustness (Anderson 2001). P-values were assessed by using a maximum of 9999 permutations of the residuals under a reduced model and critical significance levels were set, a priori, at 5%. A two-factor PERMANOVA was performed on the response variables of initial fronds (Chl *a* fluorescence: F_v/F_m, ETR_{max}, E_k, α; pigments: Chl *a*, Chl *c*, Fuc, β-caro, xanthophyll cycle pigment pool (VAZ), DPS; phlorotannins; antioxidants; total lipids, saturated/unsaturated FA ratio) to test for the effects of the factors sampling time (two levels: Jan/Feb and Aug/Sept; fixed) and sampling depth (two levels: 0.2 and 4 m; fixed). The response variables of experimental fronds exposed to 14-day transplantation (see above) were analyzed by a three-factor PERMANOVA to examine the effects of sampling time, sampling depth and the additional factor incubation depth (three levels: 0.2, 2 and 4 m; fixed). In case of a significant interaction between all three factors, a posteriori pair-wise comparisons among all pairs of levels of incubation depth were conducted. As PERMANOVA is sensitive to within-factor differences in multivariate dispersions, a permutational analysis of multivariate dispersion (PERMDISP) test was performed for each factor to ensure that multivariate dispersions between groups are homogeneous. Similarity percentage analyses (SIMPER) were carried out to identify the three response variables that contributed most to differences in long- and short-term acclimation patterns. Additionally, principal coordinate analyses (PCO) were used to visualize these differences graphically. Based on the results of the SIMPER analyses, response variables mainly driving the differences were included as overlaying vectors in the plots. Due to differences in units and scales of response variables, all data were normalized prior to analyses. Normalization was done by subtracting the mean from each single variable and dividing this variable by its respective standard deviation. All statistical tests were performed on initial and experimental fronds separately by using PRIMER v6 with PERMANOVA+ add-on (PRIMER-E Ltd; Plymouth, UK).

RESULTS

Abiotic conditions at various water depths

Differences in PAR amongst incubation depths were not consistent with time (two-way ANOVA, Ts*Di interaction, $F_{2,279}$=37.446, p<0.01). Regardless of the sampling time, a post-hoc Tukey’s HSD test displayed significant differences in levels of PAR between the three incubation depths (each p<0.001). PAR decreased to approximately 40% at 2 m and to 20% at 4 m compared to values measured near the sea surface (two-way ANOVA, main effect of incubation depth, $F_{2,279}$=290.568, p<0.001). At all three water depths, irradiance in Aug/Sept (austral winter) was 50% lower than in Jan/Feb (austral summer; two-way ANOVA, main effect of sampling time, $F_{1,279}$=227.172, p<0.001). Light attenuation (K_d) was similar in both sampling times (Student’s t test, t_{93}=-2.143, p=0.053). The differences in seawater temperature between incubation depths were not consistent over time (two-way ANOVA,

Ts*Di interaction, $F_{1,14276}$=597.210, p<0.001). Temperature followed a seasonal pattern, with higher values during Jan/Feb compared to Aug/Sept (two-way ANOVA, main effect of sampling time, $F_{1,14276}$=18938.275, p<0.001). However, due to the influence of the Humboldt Current System only minor, but significant depth-related differences in seawater temperatures were observed (two-way ANOVA, main effect of incubation depth, $F_{1,14276}$=1113.983, p<0.001; Table 4.1).

Acclimation responses to long-term depth exposure

The differences in the suite of response variables between sampling depths were not consistent over time (two-factor PERMANOVA, Ts*Ds interaction, pseudo-$F_{1,16}$=3.000, p=0.028; Table S4.3a in the Supporting Information). The PCO plot reflected the results of the PERMANOVA, with the first two axes (PCO1 and PCO2) explaining more than 60% of variation in the data (Figure 4.2a). The plot highlighted a separation of initial fronds, acclimated to the two water depths at both sampling times, with fronds from Jan/Feb 0.2 m, Aug/Sept 0.2 m and Aug/Sept 4 m showing slight overlapping, while fronds from Jan/Feb 4 m were clearly separated from the others (Figure 4.2a). The SIMPER analysis (and the vector overlays) indicated that observed differences between both sampling depths were mainly due to variation in variable Chl *a* fluorescence of PSII (ETR_{max}, E_k, α), Chl *c*, DPS and phlorotannins (Table S4.4a in the Supporting Information and Figure 4.2a). In addition to those parameters, mainly total lipids and saturated/unsaturated (sat/unsat) FA ratio seemed to drive differences in long-term acclimation to various water depths between both sampling times (Table S4.4b and Figure 4.2a). This is also obvious from individual comparisons of the respective response variables between the different sampling times and depths, with initial fronds displaying higher values of ETR_{max} and E_k and lower Chl *c* contents at 0.2 m depth than at 4 m (Tables 4.2 and 4.3). Further, initial fronds from 0.2 m in Aug/Sept showed two times higher soluble phlorotannin contents, those from 4 m in Jan/Feb and those from 0.2 m in Jan/Feb exhibited one and a half times higher total lipid contents and sat/unsat FA ratios than all other fronds, respectively (Figure 4.3a, c and d). Although levels of other photosynthetic pigments and antioxidants also differed in initial fronds from various sampling times and depths (Table 4.3 and Figure 4.3b), these response variables contributed only little to the observed differences in long-term acclimation responses (Table S4.4a and b and Figure 4.2a).

With respect to the FA composition of initial kelp fronds, 15 different FAs (five saturated and ten unsaturated FAs) were detected. FA 16:0 was the most abundant saturated FA and FA 20:4(n-6) the dominant unsaturated FA. Other principal FAs were 14:0, 18:1(n-9), 18:4(n-3) and 20:5(n-3) (for details see Table S4.5 in the Supporting Information).

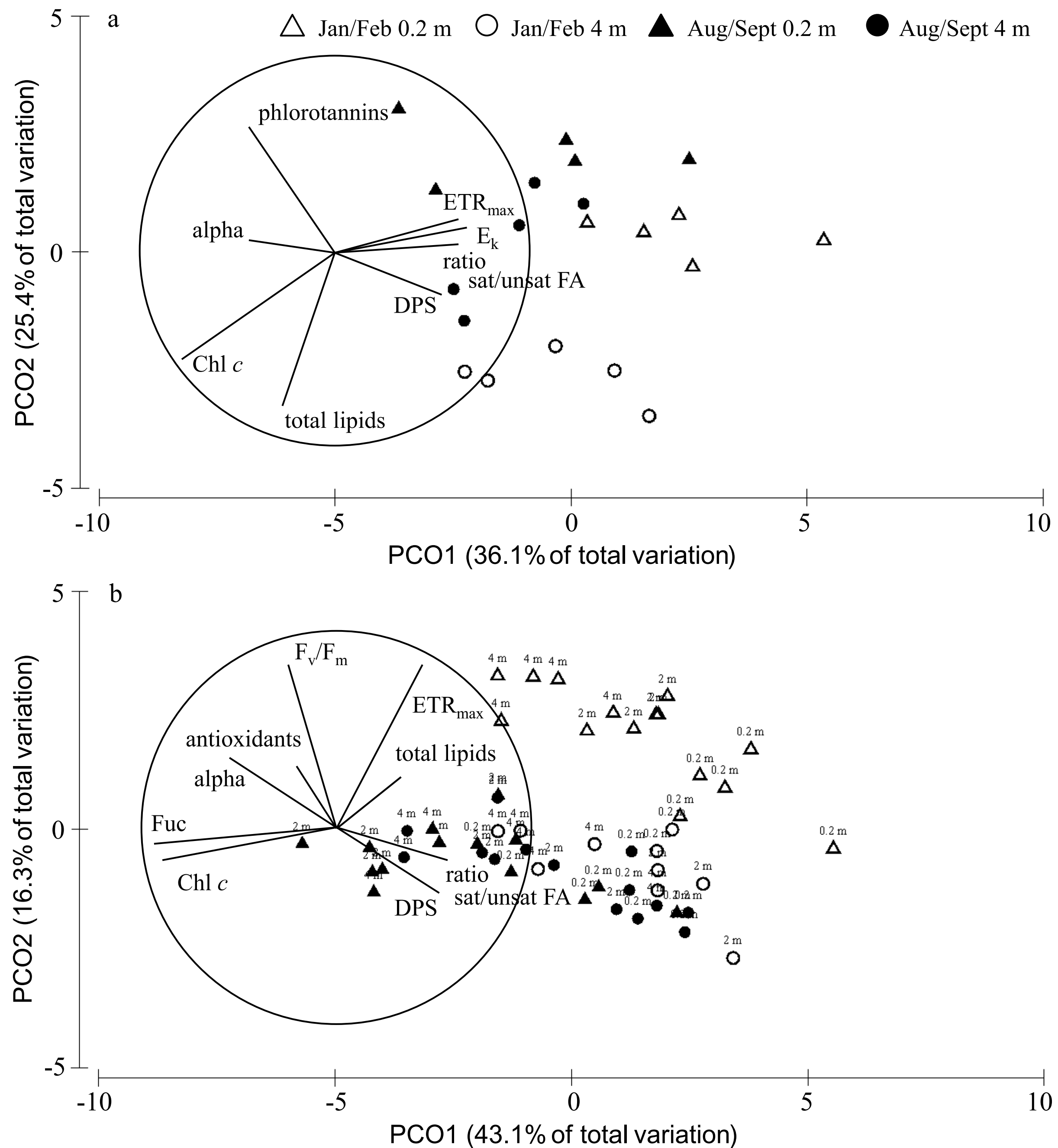

Figure 4.2: Principal coordinate analysis (PCO) plots for **(a)** initial fronds (Day 0) and **(b)** experimental fronds (Day 14) of *Macrocystis pyrifera*, based on Euclidean distances. Labels above the symbols indicate incubation depths (0.2, 2, and 4 m), to which experimental fronds were exposed for 14 days. Vector overlays represent the response variables (alpha, initial linear slope of photosynthesis versus irradiance curve; antioxidants; Chl *c*; DPS, de-epoxidation state of xanthophyll cycle pigments; E_k, saturating irradiance; ETR_{max}, maximum electron transport rate; Fuc, fucoxanthin; F_v/F_m, maximum quantum yield; phlorotannins; ratio sat/unsat FA, saturated/unsaturated fatty acid ratio; total lipids) mainly driving the observed differences, according to SIMPER analyses (Table S4.4 in the Supporting Information).

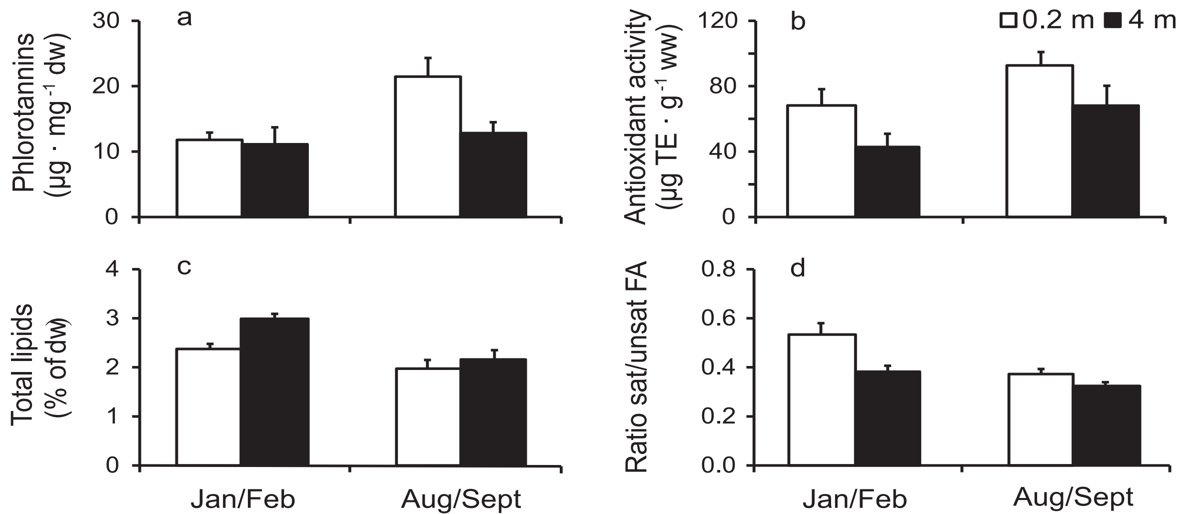

Figure 4.3: (a) Soluble phlorotannin contents (µg mg^{-1} dw), **(b)** antioxidant activities (µg TE g^{-1} ww), **(c)** total lipid contents (% of dw) and **(d)** saturated/unsaturated fatty acid ratios (ratio sat/unsat FA) of initial fronds of *Macrocystis pyrifera*, collected at two different water depths (0.2 and 4 m) during January/February (Jan/Feb) and August/September (Aug/Sept) 2012. Figure shows means±SEM (n=5).

Acclimation responses to short-term depth exposure

During the transplantation experiment, experimental kelp fronds, sampled at 0.2 m or 4 m in Jan/Feb or Aug/Sept, were able to survive and showed positive growth, but clear patterns in relation to the three incubation depths were absent. Differences in the suite of response variables amongst incubation depth were not consistent with initial sampling depth or time (three-factor PERMANOVA, Ts*Ds*Di interaction, pseudo-$F_{2,41}$=2.084, p=0.019; Table S4.3b). An a posteriori pair-wise comparison based on permutation for this interaction among all pairs of the three-level factor incubation depth displayed differences between fronds from various sampling times and depths (Table S4.6 in the Supporting Information). Kelp fronds collected at 0.2 m depth in Jan/Feb showed significant differences after transplantation along the depth gradient for 14 days among all possible pairs of incubation depth (0.2 m vs. 2 m, 0.2 m vs. 4 m and 2 m vs. 4 m). In contrast, the experimental fronds originating from 4 m depth in Jan/Feb differed only significantly between the most extreme incubation depths (0.2 m vs. 4 m). Kelp fronds from both 0.2 m and 4 m in Aug/Sept revealed significant differences between shallow waters (0.2 m) and the two deeper incubation depths (0.2 m vs. 2 m and 0.2 m vs. 4 m; Table S4.6). The PCO plot, which explained approximately 60% of the variation in the data in the two-dimensional space (PCO1 and PCO2), illustrates the results of the pair-wise comparisons (Figure 4.2b). Consequently, experimental fronds collected at 0.2 m depth in Jan/Feb were clearly distinguishable from the other fronds and revealed a distinct separation with respect to acclimation to the three incubation depths. All

other experimental fronds were grouped closer together and showed a less clear separation by acclimation among the incubation depths (Figure 4.2b).

The SIMPER analysis (and the vector overlays) suggested that differences among acclimation to incubation depths in Jan/Feb were mainly due to changes in variable Chl *a* fluorescence (F_v/F_m, α), DPS and antioxidants, whereas those in Aug/Sept resulted mainly from changes in pigments (Chl *c*, Fuc), DPS and sat/unsat FA ratio (Table S4.4c and Figure 4.2b). Changes in variable Chl *a* fluorescence (F_v/F_m, ETR_{max}), DPS, antioxidants, total lipids, and sat/unsat FA ratio accounted further for the greatest proportion of differences in short-term acclimation along the depth gradient between both sampling times (Table S4.4d and Figure 4.2b). This can also be seen by one-to-one comparisons of the respective response variables between the various incubation depths, with experimental fronds from surface waters exhibiting lower Chl *c* and Fuc contents and higher DPS than those from deeper waters (Table 4.3 and Table S4.1). Further, experimental fronds, transplanted from 4 to 0.2 m depth in Jan/Feb, showed an almost two and a half-fold increase in antioxidant activity compared to initial values (Figures 4.1b and 4.3c). Related to the sat/unsat FA ratio, the strongest acclimation response was found in kelp fronds collected at 4 m depth in Aug/Sept. The sat/unsat FA ratio of these fronds was highest at 0.2 m incubation depth and decreased with increasing depth (Figure 4.1h). In addition to the change in the degree of FA saturation, an alteration of FA chain length was observed. Experimental kelp fronds, transplanted from 4 to 0.2 m depth in Aug/Sept, exhibited a decrease in shorter-chain FAs (14, 15 and 16 C atoms) and an increase in longer-chain FAs (17, 18 and 20 C atoms) during the experimental duration of 14 days (Figure 4.4). Although amounts of other photosynthetic pigments and phlorotannins also varied in experimental fronds with respect to incubation depth (Table 4.3 and Table S4.1 and Figure 4.1a and b), these parameters did not primarily account for the observed differences in short-term acclimation responses (Table S4.4c and d and Figure 4.2b).

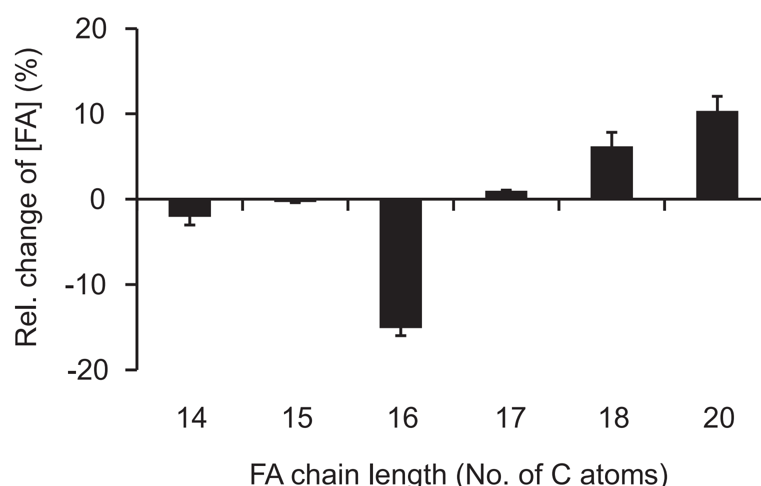

Figure 4.4: Relative change in fatty acid (FA) concentrations (%), grouped by different chain lengths, of experimental fronds of *Macrocystis pyrifera*, transplanted from 4 to 0.2 m depth for 14 days during August/September 2012. Figure shows means±SEM (n=4-5).

DISCUSSION

Overall, the results of this study confirm the high acclimation potential of *M. pyrifera* to the prevailing abiotic conditions along a depth gradient, but they should be interpreted with the understanding that depth effects are potentially influenced by spatial differences in origin of the experimental source plants. We observed both advantageous acclimation strategies to various water depths between different sampling times (long-term acclimation responses) as well as more rapid physiologically favorable adjustments during 14 days of transplantation to different depths (short-term acclimation responses). However, in contrast to other studies (e.g., 0-9 m in Gerard 1986 or 0-18 m in Colombo-Pallotta et al. 2006), we worked with a comparatively small depth gradient (0.2-4 m). Interestingly, the physiological adjustments of *M. pyrifera* were evident even at this smaller scale, which further illustrates the enormous acclimation capacity of this kelp species.

Acclimation responses to long-term depth exposure

With respect to long-term acclimation responses, initial fronds of *M. pyrifera*, growing at various water depths in Jan/Feb or Aug/Sept, exhibited different physiological acclimation states due to the respective conditions of solar radiation and temperature. Statistical analyses suggested that adjustments in variable Chl *a* fluorescence of PSII, photosynthetic pigments, phlorotannins, total lipids, and the degree of FA saturation mainly contributed to these observed differences. Initial fronds followed the well-known pattern of photoacclimation to various light conditions along the depth gradient by displaying higher values of ETR_{max} and E_k and lower concentrations of the antenna pigment Chl *c* in shallow than in deep waters

(Wheeler 1980, Smith and Melis 1987, Reiskind et al. 1989). Thus, photosynthesis of *M. pyrifera* occurring in greater water depths is already saturated at lower light intensities (Reiskind et al. 1989) and the observed variations in Chl *c* concentrations allow the alga to absorb more photons under lower light intensities in deeper waters (e.g., Wheeler 1980, Smith and Melis 1987). Consequently, the observed acclimation responses are beneficial for *M. pyrifera* growing under the different environmental conditions at various water depths with different light regimes in Jan/Feb and Aug/Sept.

Higher soluble phlorotannin contents of initial fronds from 0.2 m depth compared to 4 m in Aug/Sept might indicate the protective role of these compounds against the adverse effects of high solar radiation near the water surface (Schoenwaelder 2002). Surprisingly, this finding could only be observed in fronds from Aug/Sept but not in those from Jan/Feb and is in contrast to the seasonal pattern of phlorotannin levels found in the other large kelp, *Durvillaea antarctica*, which frequently occurs together with *M. pyrifera* along the Chilean coastline (Tala et al. 2013). Why *M. pyrifera* reacted contrarily to this often observed pattern remains unresolved.

Initial fronds probably growing under lower water temperatures and solar radiation (Jan/Feb 4 m, Aug/Sept 0.2 and 4 m) exhibited one and a half times lower sat/unsat FA ratios than those exposed to the highest temperature and solar radiation levels (Jan/Feb 0.2 m). We suggest that this enhanced degree of unsaturation might act as compensation for the decrease in membrane fluidity, which is induced by low temperature and possibly also low light (Buchanan et al. 2000). Increased FA desaturation as a response to a drop in temperature has been observed already in several brown algal species (e.g., *Fucus vesiculosus*, *Laminaria japonica*, *Sargassum pallidum*; Pohl and Zurheide 1979, Sanina et al. 2008) and those changes with respect to differences in abiotic conditions play a key role in the readjustment of cellular membranes, so that these can remain operative (Thompson 1996). This is especially important for thylakoid membranes, which contain the photosynthetic apparatus. To maintain a high photosynthetic capacity, degraded proteins must be removed and *de novo* synthesized proteins must be re-integrated into the reaction centers (i.e., D1 protein turnover). This process takes place via lateral diffusion through the thylakoid membranes and is highly dependent on membrane fluidity (Becker et al. 2010 and references therein).

Acclimation responses to short-term depth exposure

Overall, after 14 days of transplantation along a depth gradient, experimental fronds of *M. pyrifera* collected at various sampling times and depths adjusted their physiological states to the new abiotic conditions, resulting in differences in short-term acclimation responses to solar radiation and temperature levels along the depth gradient. These results should be

interpreted with the understanding that multiple abiotic factors might have influenced the acclimation responses of *M. pyrifera* simultaneously. In this study, we only concentrated on differences in irradiance of PAR and water temperature along the depth gradient. Other factors, like nutrient concentrations, could also vary at the various water depths and thereby affect algal ecophysiology (Buschmann et al. 2014b), although previous studies did not find nitrogen limitation in sporophytes of *M. pyrifera* in waters of northern-central Chile (e.g., Rothäusler et al. 2011b), which are rich in nutrients, particularly nitrogen, without clear vertical gradients (Moraga and Olivares 1993). In particular, kelp fronds collected at 0.2 m in Jan/Feb developed physiological states that varied clearly between all three incubation depths. We propose that this differential acclimation might be due to the maximum amplitude of changing solar radiation and temperature conditions along the depth gradient in Jan/Feb (Table 4.1). In contrast, kelp fronds from 4 m sampling depth in Jan/Feb did not show this pronounced differentiation after transplantation, despite experiencing the same amplitude of change in abiotic conditions, only in the opposite direction. We presume that transient stress caused by photoinhibition right after transplantation from deep (4 m) to surface (0.2 m) solar radiations, as indicated by measurements of F_v/F_m on day 7 of the experiment (data not shown), might be responsible for this observation. This interpretation is supported by the almost two and a half-fold increase in antioxidant activity, which also is plausible, because antioxidants are known to protect the photosynthetic apparatus against permanent photodamage by scavenging ROS, formed at the photosynthetic electron transport chain under intense solar radiation conditions (Bischof and Rautenberger 2012). Similar results were reported in a study by Cruces et al. (2012) on the effects of temperature and UV on the radical scavenging activity in South Pacific kelps. They reported maximum scavenging activity after UV-exposure at 20°C. These abiotic conditions are comparable to those prevailing in surface waters during Jan/Feb in our study.

Our results suggested that differences in short-term acclimation responses were mainly driven by changes in Chl *a* fluorescence of PSII, photosynthetic pigments, total lipids, and the degree of FA saturation in addition to the shifts in antioxidant activities. This response was similar to the adjustments found during long-term acclimation, in that experimental fronds responded according to the classical pattern of photoacclimation along a depth gradient by exhibiting increased Chl *c* and Fuc contents at greater water depths in order to adjust antenna sizes to the new prevailing irradiances (e.g., Wheeler 1980, Smith and Melis 1987). Contrarily, de-epoxidation states of the xanthophyll cycle pigments were greatest in shallow waters and decreased with depth. Similar results were detected by Colombo-Pallotta et al. (2006) and García-Mendoza and Colombo-Pallotta (2007). Since xanthophyll cycle pigment de-epoxidation can be activated within several minutes, this protection mechanism of the photosynthetic apparatus in terms of excess light energy dissipation via NPQ might be

especially important during abrupt changes of potentially stressful environmental conditions (Yamamoto et al. 1962), which are more pronounced in surface waters than at greater depths (e.g., Gerard 1984, Hanelt 1996). Additionally, this protection mechanism would be especially important when algae detach from the primary substratum and float to the sea surface (Rothäusler et al. 2011a). However, although shifts in variable Chl *a* fluorescence of PSII accounted for a great proportion of differences in short-term acclimation, these parameters were quite variable and did not show a consistent trend according to incubation depth. The susceptibility of PAM fluorometry to various factors (e.g., variable weather conditions during measuring days, use of different blade parts for measurements due to grazing effects or losses of replicates due to strong wave action) might be responsible for the observed variability (Edwards and Kim 2010). Overall, high maximum quantum yields (approximately 90-115% of initial values) indicated that the kelp fronds were not continuously stressed and showed no signs of photodamage on day 14 of the experiments. However, *M. pyrifera*, which had been sampled at 4 m depth, showed unexpectedly high increases of ETR_{max} and E_k (approximately 4- to 7-fold increases) after transplantation to the three water depths in Jan/Feb and Aug/Sept. One possible explanation might be the loss of self-shading and continuous vertical displacement of blade layers, which typically occur within a kelp forest (Gerard 1986), during our transplantation experiment.

Experimental fronds responded to the transplantation from 4 m to 0.2 m water depth with a rise in the degree of FA saturation (2-fold increase of sat/unsat FA ratio) and a switch from shorter- to longer-chain FAs. Interestingly, this strong response occurred only in Aug/Sept, but not in Jan/Feb. One possible explanation for this finding is that in Aug/Sept the kelp had to cope with a particularly challenging combination of abiotic conditions (high PAR at low temperature) in surface waters. Since during Aug/Sept, temperature barely changed with water depth, we suggest that differences in PAR levels might be the major factor for the observed changes in FA composition, but this assumption has to be verified in further laboratory studies (see Conclusions and outlook). Although light-induced variation in FAs of macroalgae is a frequently observed phenomenon (e.g., Pettitt and Harwood 1989, Levy et al. 1992), its implication is not entirely clear and less plausible than the temperature-induced sensitivity of membrane fluidity.

CONCLUSIONS AND OUTLOOK

Macrocystis pyrifera was capable of adjusting its physiological status to various conditions of solar radiation and temperature along a depth gradient on a long-term and short-term timescale, in which the latter might be especially important in the context of detachment and rafting. Our results indicated that acclimation strategies in *M. pyrifera* can vary on different temporal scales, with phlorotannins being important during long-term acclimation and

antioxidants playing a crucial role during short-term acclimation. Furthermore, adjustments in total lipids and FA compositions apparently contribute to acclimation strategies to an extent comparable to other response variables (variable Chl *a* fluorescence, pigments, phlorotannins, antioxidants). To our knowledge, this is the first study showing the importance of lipid adjustments for acclimation in *M. pyrifera*. However, with the chosen experimental set-up, it is difficult to disentangle which abiotic factor (solar radiation, temperature, or others) is responsible for the observed physiological changes, especially with respect to FA composition. Future fully controlled field or laboratory experiments are needed to elucidate the relative influences of the abiotic parameters. Further, future studies should focus on changes in FA composition within different lipid classes of *M. pyrifera*, which would allow identification of which specific membranes (e.g., thylakoid membranes) are primarily affected by the observed adjustments in FAs.

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SUPPORTING INFORMATION

Table S4.1: Pigment concentrations (µg mg^{-1} dw) and DPS of experimental fronds of *Macrocystis pyrifera* (Day 14) from two different sampling depths (0.2 and 4 m), transplanted to 2 m water depth during January/February (Jan/Feb) and August/September (Aug/Sept) 2012.

Pigment	Sampling depth (m)	Incubation depth (m)	Jan/Feb 2012 Day 14	Aug/Sept 2012 Day 14
Chl *a*	0.2	2	1.26±0.08	2.78±0.28
	4	2	1.33±0.17[a]	1.90±0.12
Chl *c*	0.2	2	0.08±0.02	0.29±0.04
	4	2	0.13±0.02[a]	0.20±0.02
Fuc	0.2	2	0.43±0.03	1.03±0.12
	4	2	0.52±0.07[a]	0.75±0.06
β-caro	0.2	2	0.08±0.01	0.15±0.01
	4	2	0.06±0.01[a]	0.10±0.01
VAZ	0.2	2	0.12±0.01	0.26±0.02
	4	2	0.10±0.02[a]	0.17±0.01
DPS	0.2	2	0.066±0.002	0.082±0.021
	4	2	0.110±0.029[a]	0.061±0.017

Additionally, experimental fronds were also transplanted to 0.2 and 4 m water depth. Those data are given in Table 4.3. Table shows means±SEM (n=5, with exception of [a]n=4).
β-caro, β-carotene; DPS, de-epoxidation state of xanthophyll cycle pigments; Fuc, fucoxanthin; SEM, standard error of the mean; VAZ, xanthophyll cycle pigment pool.

Table S4.2: Characteristics of variable Chl *a* fluorescence of PSII of experimental fronds of *Macrocystis pyrifera* (Day 14) from two different sampling depths (0.2 and 4 m), transplanted to 2 m water depth during January/February (Jan/Feb) and August/September (Aug/Sept) 2012.

Parameter of Chl *a* fluorescence	Sampling depth (m)	Incubation depth (m)	Jan/Feb 2012 Day 14 (% of initial)	Aug/Sept 2012 Day 14 (% of initial)
F_v/F_m	0.2	2	111±2	101±1
	4	2	94±4[a]	104±3
ETR_{max}	0.2	2	212±15	106±18
	4	2	451±11[a]	177±26
E_k	0.2	2	173±22	104±21
	4	2	757±78[a]	147±22
α	0.2	2	126±9	102±3
	4	2	58±5[a]	105±7

Additionally, experimental fronds were also transplanted to 0.2 and 4 m water depth. Those data are given in Table 4.2. Different PAM devices were used for the measurements in Jan/Feb and Aug/Sept. For a better comparability between both sampling times, the values are expressed as % of initial. Table shows means±SEM (n=5, with exception of [a]n=4).

α, initial linear slope of photosynthesis versus irradiance curve; E_k, saturating irradiance; ETR_{max}, maximum electron transport rate; F_v/F_m, maximum quantum yield; SEM, standard error of the mean.

Table S4.3: Results of the statistical analysis of response variables (F_v/F_m, ETR_{max}, E_k, α, Chl *a*, Chl *c*, Fuc, β-caro, VAZ, DPS, phlorotannins, antioxidants, total lipids, saturated/unsaturated FA ratio) from **(a)** initial fronds of *Macrocystis pyrifera* (Day 0), using a two-factor permutational multivariate analysis of variance (PERMANOVA) with the factors sampling time (Ts: January/February and August/September), sampling depth (Ds: 0.2 and 4 m) and their interactions and **(b)** experimental fronds of *M. pyrifera* (Day 14), using a three-factor PERMANOVA with the factors sampling time (Ts: January/February and August/September), sampling depth (Ds: 0.2 and 4 m), incubation depth (Di: 0.2, 2 and 4 m) and their interactions.

	(a) Initial fronds (Day 0)				(b) Experimental fronds (Day 14)			
	df	MS	pseudo-F-ratio	p-value	df	MS	pseudo-F-ratio	p-value
Ts	1	57.878	7.050	**<0.001**	1	132.510	23.123	**<0.001**
Ds	1	52.135	6.350	**<0.001**	1	62.721	10.945	**<0.001**
Di					2	53.963	9.416	**<0.001**
Ts*Ds	1	24.631	3.000	**0.028**	1	54.921	9.584	**<0.001**
Ts*Di					2	24.302	4.241	**<0.001**
Ds*Di					2	11.269	1.966	**0.028**
Ts*Ds*Di					2	11.943	2.084	**0.019**
Residual	16	8.210			41	5.731		
Total	19				52			

P-values in bold highlight significant differences at $p<0.05$.

α, initial linear slope of photosynthesis versus irradiance curve; β-caro, β-carotene; DPS, de-epoxidation state of xanthophyll cycle pigments; E_k, saturating irradiance; ETR_{max}, maximum electron transport rate; FA, fatty acid; Fuc, fucoxanthin; F_v/F_m, maximum quantum yield; VAZ, xanthophyll cycle pigment pool.

Table S4.4: Results from similarity percentage analyses (SIMPER) showing the relative contribution (%) of single response variables to differences in long- and short-term acclimation. **(A)** Long-term acclimation: **(a)** effect of sampling depth: comparison of long-term acclimation to shallow (0.2 m) and deep (4 m) sampling depths within one sampling time and **(b)** effect of sampling time: comparison of long-term acclimation to shallow (0.2 m) and deep (4 m) sampling depths between both sampling times. **(B)** Short-term acclimation: **(c)** effect of incubation depth: comparison of short-term acclimation between shallow (0.2 m) and deep (4 m) incubation depths within one sampling time and **(d)** effect of sampling time: comparison of short-term acclimation to shallow (0.2 m) and deep (4 m) incubation depths between both sampling times. For short-term acclimation, comparisons are only given for the two extreme incubation depths (0.2 and 4 m).

(A) Comparison (Ts Ds ↔ Ts Ds)	**Main contributors to differences in long-term acclimation (%)**	
(a) Effect of sampling depth		
Jan/Feb 0.2 m ↔ Jan/Feb 4 m	Chl *c*	11.8
	E_k	11.6
	ETR_{max}	11.1
Aug/Sept 0.2 m ↔ Aug/Sept 4 m	Phlorotannins	14.0
	DPS	13.4
	α	9.6
(b) Effect of sampling time		
Jan/Feb 0.2 m ↔ Aug/Sept 0.2 m	E_k	11.5
	Ratio sat/unsat FA	10.4
	Phlorotannins	9.9
Jan/Feb 4 m ↔ Aug/Sept 4 m	Total lipids	14.5
	DPS	13.2
	α	10.5
(B) Comparison (Ts Ds Di ↔ Ts Ds Di)	**Main contributors to differences in short-term acclimation (%)**	
(c) Effect of incubation depth		
Jan/Feb 0.2 m 0.2 m ↔ Jan/Feb 0.2 m 4 m	α	21.9
	F_v/F_m	18.9
	DPS	11.9
Jan/Feb 4 m 4 m ↔ Jan/Feb 4 m 0.2 m	DPS	24.9
	Antioxidants	16.9
	α	11.7
Aug/Sept 0.2 m 0.2 m ↔ Aug/Sept 0.2 m 4 m	Fuc	16.6
	Chl *c*	13.9
	DPS	13.8
Aug/Sept 4 m 4 m ↔ Aug/Sept 4 m 0.2 m	Ratio sat/unsat FA	21.9
	Fuc	13.5
	Chl *c*	13.2
(d) Effect of sampling time		
Jan/Feb 0.2 m 4 m ↔ Aug/Sept 0.2 m 4 m	ETR_{max}	22.6
	F_v/F_m	18.5
	Total lipids	10.5
Jan/Feb 4 m 0.2 m ↔ Aug/Sept 4 m 0.2 m	DPS	24.6
	Ratio sat/unsat FA	21.9
	Antioxidants	13.6

Table shows the three response variables, which account mainly for differences in long- and short-term acclimation responses.

α, initial linear slope of photosynthesis versus irradiance curve; Aug/Sept, August/September; Di, incubation depth (0.2 and 4 m); DPS, de-epoxidation state of xanthophyll cycle pigments; Ds, sampling depth (0.2 and 4 m); E_k, saturating irradiance; ETR_{max}, maximum electron transport rate; Fuc, fucoxanthin; F_v/F_m, maximum quantum yield; Jan/Feb, January/February; ratio sat/unsat FA, saturated/unsaturated fatty acid ratio; Ts, sampling time (Jan/Feb and Aug/Sept).

Table S4.5: Fatty acid (FA) compositions (mass % of total fatty acids) of initial fronds of *Macrocystis pyrifera*, sampled at two different water depths (0.2 and 4 m) during January/February (Jan/Feb) and August/September (Aug/Sept) 2012.

	Jan/Feb 2012		Aug/Sept 2012	
FA	**0.2 m**	**4 m**	**0.2 m**	**4 m**
14:0	8.2±0.8	7.5±0.3	9.1±0.9	7.2±0.5
15:0	0.5±0.0	0.2±0.1	0.2±0.1	0.2±0.0
16:0	22.6±1.1	17.7±1.0	16.1±0.8	15.2±0.7
16:1(n-7)	3.7±0.2	4.3±1.0	1.6±0.1	1.8±0.2
17:1(n-7)	n.d.	n.d.	1.0±0.1	1.0±0.1
18:0	1.1±0.1	0.9±0.1	0.5±0.0	0.6±0.1
18:1(n-9)	13.1±1.0	12.3±0.6	12.7±0.7	12.2±1.0
18:2(n-6)	6.2±0.4	6.1±0.3	5.1±0.2	5.5±0.2
18:3(n-6)	n.d.	0.7±0.0	0.2±0.1	0.5±0.1
18:3(n-3)	5.9±0.5	6.9±0.5	7.4±0.3	8.7±0.5
18:4(n-3)	8.3±1.0	11.3±1.4	12.5±1.0	13.3±1.2
20:0	0.6±0.0	0.4±0.2	0.2±0.1	0.4±0.1
20:4(n-6)	15.0±0.9	16.0±1.2	17.4±0.4	17.3±0.7
20:4(n-3)	0.9±0.1	1.1±0.1	0.6±0.0	0.9±0.1
20:5(n-3)	9.3±0.9	11.4±0.8	11.6±0.6	11.7±0.3

Table shows means±SEM (n=5).
n.d., not detected; SEM, standard error of the mean.

Table S4.6: Results of pair-wise comparisons based on permutation of significant interaction of the factors *Sampling time x Sampling depth x Incubation depth* on response variables (F_v/F_m, ETR_{max}, E_k, α, Chl *a*, Chl *c*, Fuc, β-caro, VAZ, DPS, phlorotannins, antioxidants, total lipids, saturated/unsaturated FA ratio) from experimental fronds of *Macrocystis pyrifera* (Day 14).

Sampling time	Sampling depth	Pair-wise comparisons of incubation depths	t-value	p-value
Jan/Feb	0.2 m	0.2 m vs. 2 m	2.826	**0.008**
		0.2 m vs. 4 m	4.009	**0.008**
		2 m vs. 4 m	2.137	**0.016**
Jan/Feb	4 m	0.2 m vs. 2 m	1.429	0.096
		0.2 m vs. 4 m	1.950	**0.047**
		2 m vs. 4 m	2.064	0.053
Aug/Sept	0.2 m	0.2 m vs. 2 m	2.905	**0.008**
		0.2 m vs. 4 m	2.133	**0.014**
		2 m vs. 4 m	1.396	0.128
Aug/Sept	4 m	0.2 m vs. 2 m	2.053	**0.017**
		0.2 m vs. 4 m	2.031	**0.016**
		2 m vs. 4 m	0.774	0.736

Pair-wise comparisons were conducted among all pairs of levels of the factor incubation depth. P-values in bold highlight significant differences at $p<0.05$.
α, initial linear slope of photosynthesis versus irradiance curve; Aug/Sept, August/September; β-caro, β-carotene; DPS, de-epoxidation state of xanthophyll cycle pigments; E_k, saturating irradiance; ETR_{max}, maximum electron transport rate; FA, fatty acid; Fuc, fucoxanthin; F_v/F_m, maximum quantum yield; Jan/Feb, January/February; VAZ, xanthophyll cycle pigment pool.

5 PUBLICATION III

FATTY ACID COMPOSITIONS ASSOCIATED WITH HIGH-LIGHT TOLERANCE IN THE INTERTIDAL RHODOPHYTES *MASTOCARPUS STELLATUS* AND *CHONDRUS CRISPUS*

Kristina Koch[1,2], Wilhelm Hagen[2,3], Martin Graeve[4] and Kai Bischof[1,2]

[1]Marine Botany, University of Bremen, Bremen, Germany
[2]Bremen Marine Ecology – Center for Research and Education (BreMarE), University of Bremen, Bremen, Germany
[3]Marine Zoology, University of Bremen, Bremen, Germany
[4]Ecological Chemistry, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany

Helgoland Marine Research (minor revision)

ABSTRACT

The rhodophytes *Mastocarpus stellatus* and *Chondrus crispus* occupy the lower intertidal zone of rocky shores along North Atlantic Ocean coastlines, with *C. crispus* generally occurring slightly deeper. Consequently, *M. stellatus* is exposed to more variable environmental conditions, related to a generally higher stress tolerance of this species. In order to extend our understanding of seasonal modulation of stress tolerance, we subjected *M. stellatus* and *C. crispus* from Helgoland, North Sea, to short-term high-light stress experiments over the course of a year (October 2011, March, May and August 2012). Biochemical analyses (pigments, antioxidants, total lipids, fatty acid composition) allowed to reveal mechanisms behind modulated high-light tolerances. Overall, *C. crispus* was particularly more susceptible to high-light at higher water temperatures (October 2011 and August 2012). Furthermore, species-specific differences in antioxidants, total lipids and the shorter-chain/longer-chain fatty acid ratio (C14+C16/C18+C20) were detected, which might enhance the tolerance to high-light and other abiotic stress factors in *M. stellatus*, so that this species is more competitive in the highly variable upper intertidal zone compared to *C. crispus*. Since the high-light tolerance in *C. crispus* seemed to be affected by water temperature, the competition between both species might be modulated in the future under rising mean annual sea surface temperature around the island of Helgoland.

KEYWORDS

Antioxidants, *Chondrus crispus*, Helgoland, high-light stress, fatty acid composition, *Mastocarpus stellatus*

ABBREVIATIONS

ANOVA, analysis of variance; Aug, August; BAH, Biological Institute Helgoland; C14, fatty acids with 14 carbon atoms; C16, fatty acids with 16 carbon atoms; C18, fatty acids with 18 carbon atoms; C20, fatty acids with 20 carbon atoms; C14+C16/C18+C20, shorter-chain/longer-chain fatty acid ratio; Chl *a*, chlorophyll *a*; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; DPPH, 2,2-diphenyl-1-picrylhydrazyl; E_k, saturating irradiance; ETR, electron transport rate; F_0, dark adapted initial minimal fluorescence; FAME, fatty acid methyl ester derivate; F_m, maximal fluorescence; F_v, variable fluorescence; F_v/F_m, maximum quantum yield; HPLC, high performance liquid chromatography; LED, light-emitting diode; LSD, least significant difference; MAA, mycosporine-like amino acid; Mar, March; Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; MUFA, sum of monounsaturated fatty acids; Oct, October; PAM, pulse amplitude modulation; PAR, photosynthetically active radiation; P-E curve, photosynthesis versus irradiance curve; PSII, photosystem II; PUFA, sum of polyunsaturated fatty acids; ROS, reactive oxygen species; SEM, standard error of the mean; SFA, sum of saturated fatty acids; SFA/UFA, saturated/unsaturated fatty acid ratio; TE, Trolox equivalent; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; UV, ultraviolet radiation

INTRODUCTION

Mastocarpus stellatus ((Stackhouse) Guiry, 1984; Gigartinales, Rhodophyta) and *Chondrus crispus* (Stackhouse, 1797; Gigartinales, Rhodophyta) are closely related morphologically similar red macroalgal species, both approximately 10 cm in size with numerous dichotomously branching blades arising from a flattened stipe (McLachlan et al. 1989, Guiry and Garbary 1990, Kornmann and Sahling 1994). In the lower intertidal zone of rocky shorelines along the North Atlantic Ocean coastlines (Lüning 1990), *M. stellatus* and *C. crispus* are of significant ecological and economic importance, providing food and habitat to associated invertebrates (McLachlan 1991, Kornmann and Sahling 1994) and representing a source of carrageenan, which is used in the food, cosmetic and pharmaceutical industries (Gómez-Ordóñez et al. 2010). Additionally, the species are of commercial interest due to their high content of polyunsaturated fatty acids with 20 carbon atoms such as 20:4(n-6) (arachidonic acid) and 20:5(n-3) (eicosapentaeonic acid; Mabeau and Fleurence 1993). Arachidonic acid has medical significance as precursor of

prostaglandins, whereas eicosapentaeonic acid is an essential constituent in the feed of several mariculture species and is suggested to reduce the risk of thrombosis, atherosclerosis and heart disease in humans (Floreto et al. 1993, Ortiz et al 2009).

As inhabitants of the intertidal zone, *M. stellatus* and *C. crispus* alternate between periods of immersion in seawater and exposure to air, where they experience several potentially stressful environmental conditions such as high photosynthetically active and ultraviolet radiation (PAR and UV), high or low temperatures (e.g., changes of 10 to 20°C compared to seawater temperature in the Gulf of Maine, USA; Davison and Pearson 1996), desiccation, osmotic stress and nutrient limitation (Kübler and Davison 1993). To prevail in their particularly challenging, dynamic environment, intertidal macroalgae have generally developed effective ecophysiological acclimation mechanisms (e.g., Kübler and Davison 1993). Such mechanisms may include a high scavenging capacity for reactive oxygen species (ROS; Collén and Davison 1999, Lohrmann et al. 2004) and UV-screening substances, like mycosporine-like amino acids (MAA), commonly found in red algae (Karsten et al. 1998, Bischof et al. 2000). Furthermore, thylakoid membrane integrity needs to be maintained and adjusted to the prevailing environmental conditions to allow proper operation of the photosynthetic machinery in a highly variable environment. The respective composition of fatty acids, affecting membrane fluidity, is, thus, of particular significance to maintain photosynthetic functions (e.g., re-integration of *de novo* synthesized D1 reaction center proteins; Becker et al. 2010 and references therein). Membrane fluidity is mainly determined by the chain length of fatty acids and their saturation state. It is generally accepted that at low temperatures, biological membranes exhibit higher amounts of shorter-chain and unsaturated fatty acids with lower melting points. At high temperatures, vice versa, more longer-chain and saturated fatty acids with higher melting points are incorporated into membranes (Buchanan et al. 2000). Previous studies pointed out that changes in environmental parameters can cause alterations in fatty acid profiles of marine rhodophytes (e.g., Pettitt et al. 1989, Pettitt and Harwood 1989). Additionally, algae growing at various vertical shore levels differed in their fatty acid saturation levels (Ito and Tsuchiya 1977, Becker et al. 2010).

The frequency and duration of emersed periods during low tide depends on the vertical position of an alga on the shore. Species found higher on the coast are generally thought to be less susceptible to environmental stress than those inhabiting lower levels (Dring et al. 1996, Sagert et al. 1997, Collén and Davison 1999). *Mastocarpus stellatus* and *C. crispus* occupy different levels within the lower intertidal, with *C. crispus* generally occurring slightly deeper (Lüning 1990). Along the south-western coast of the island of Helgoland in the North Sea, for example, the highest part of the lower intertidal is dominated by an almost monospecific zone of *M. stellatus*, whereas in the deeper part the two macroalgal species co-

occur as mixed assemblages (Bartsch and Tittley 2004). Consequently, *M. stellatus* is considered as being more tolerant with respect to the adverse effects of UV-B radiation (Bischof et al. 2000), freezing (Davison et al. 1989, Dudgeon et al. 1989) and desiccation (Dudgeon et al. 1995) than *C. crispus*. Interestingly, *M. stellatus* was not recorded on Helgoland before 1983, when the species was accidentally introduced to the island during scientific field experiments. Afterwards, *M. stellatus* established and massively dispersed over the island, with drastic alterations of the native communities (Kornmann and Sahling 1994, Bartsch and Kuhlenkamp 2000). Differences in stress tolerances may be advantageous for *M. stellatus* over *C. crispus* in terms of competition and colonization of new habitats (Davison et al. 1989, Dudgeon et al. 1989, 1995, Bischof et al. 2000).

The object of the present study was to extend our understanding of stress tolerance in *M. stellatus* and *C. crispus* from Helgoland. As light exposure is a major factor controlling the vertical distribution of algae on the shore, we selected high-light as abiotic variable in stress experiments. More specifically, we tackled the question whether differences in high-light tolerance are species-specific or rather habitat-specific, with habitat being defined as vertical position on the shore. Further, we checked for the possible ecophysiological mechanisms behind different high-light tolerances. Besides measurements of pigment concentrations and antioxidant activities, we determined total lipid content and the fatty acid composition. Since solar radiation strongly varies between seasons (Dring et al. 2001), we performed our study during four events over the course of one year.

MATERIALS AND METHODS

Algal material and sampling site

Individuals of *Mastocarpus stellatus* and *Chondrus crispus* were collected during low tide at the south-western rocky shore of the island of Helgoland (German Bight, North Sea, 54°11`N, 7°53`E) during four sampling events (21 October (Oct) 2011; 7 March (Mar), 14 May and 9 August (Aug) 2012). *Mastocarpus stellatus* (hereafter isolate Mast-ex) was taken from higher levels of the lower intertidal, which were fully exposed to air during low tide. Additionally, *M. stellatus* (hereafter isolate Mast-ov) and *C. crispus* (hereafter isolate Chon-ov) were sampled from deeper levels of the lower intertidal, only exposed to air for limited times. In the latter position, both species occurred within an overlapping zone. Collected algal individuals were directly placed in plastic bags with sufficient seawater to keep them moist. Afterwards, algal individuals were kept in darkness and immediately transported to the marine laboratory of the Biological Institute Helgoland (BAH) of the Alfred Wegener Institute, where they were stored overnight in a flow-through seawater basin (approximately 100 l) at ambient water temperature (Table 5.1). One day later, algal individuals were transported in coolers under dark, cool and moist conditions to the

laboratory of the Department of Marine Botany at the University of Bremen, where the high-light stress experiment and the ecophysiological analyses were conducted.

Table 5.1: Experimental conditions applied during pre-acclimation (light-dark cycle, water temperature) and high-light stress exposure (water temperature, 10x saturating irradiance of algal photosynthesis (E_k)) of algal isolates (Mast-ex, Mast-ov and Chon-ov) from the four sampling events (Oct 2011; Mar, May and Aug 2012). E_k-values were defined by P-E curve fitting after Jassby and Platt (1976) and are given as means±SEM (n=6). High-light stress was defined as 10x E_k, so that it was possible to expose the three algal isolates from four sampling events to comparable stress conditions.

Experimental conditions	**Sampling event**			
	Oct 2011	**Mar 2012**	**May 2012**	**Aug 2012**
Light-dark cycle (h)	10:14	11:13	16:8	15:9
Water temperature (°C)	14	4	8	16
E_k (µmol photons m^{-2} s^{-1})				
Mast-ex	71.4±7.7	21.5±5.3	91.8±6.2	56.0±4.0
Mast-ov	75.7±7.9	10.4±2.6	129.9±19.5	42.5±4.6
Chon-ov	83.2±9.1	7.8±2.0	40.1±10.4	56.6±7.5
10x E_k (High-light stress; µmol photons m^{-2} s^{-1})				
Mast-ex	710	220	920	560
Mast-ov	760	100	1300	430
Chon-ov	830	80	400	570

Aug, August; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; Mar, March; Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Oct, October; P-E curve, photosynthesis versus irradiance curve; SEM, standard error of the mean

High-light stress experiment

Subsequently, algal individuals were cleaned of any visible epibionts and their holdfasts were removed, so that thallus branches of about 2 cm remained. Thallus branches were kept for 24 h in continuously aerated seawater at an irradiance of approximately 30 µmol photons m^{-2} s^{-1} (provided by daylight fluorescence tubes) at light and dark cycles and temperatures being consistent with the environmental conditions found in the field (pre-acclimation; Table 5.1).

In order to test for differences in high-light susceptibility between the three algal isolates from different shore levels, short-term responses in maximum quantum yields (F_v/F_m) were monitored with a pulse amplitude-modulated fluorometer (PAM 2500; Walz, Effeltrich, Germany) during a high-light stress experiment. Maximum quantum yields were determined in dark adapted (5 min) thallus branches and calculated as:

$$F_v/F_m = (F_m - F_0) / F_m$$

with the variable fluorescence (F_v) representing the difference between the maximal fluorescence (F_m), when all photosystem II (PSII) reaction centers are reduced, and the dark adapted initial minimal fluorescence (F_0), when all PSII reaction centers are oxidized (Schreiber et al. 1994).

High-light stress was defined as 10x the saturating irradiance of algal photosynthesis (E_k), so that it was possible to expose the three algal isolates from four sampling events to comparable stress conditions (Table 5.1). Prior to the experiment, electron transport rates (ETR; 6 replicates per isolate) were estimated from rapid photosynthesis versus irradiance curves (P-E curves). Thallus branches were irradiated with a series of stepwise increasing actinic irradiances (approximately 20-1800 µmol photons m^{-2} s^{-1}) at 30 s intervals, provided by a red light-emitting diode (LED; Schreiber et al. 1994). Subsequently, the saturating irradiance was defined by P-E curve fitting after Jassby and Platt (1976) (Table 5.1).

For the experiment, thallus branches were placed in glass crystallizing dishes (diameter: 10 cm) filled with approximately 100 ml filtered (pore size: 0.2 µm) seawater at ambient temperature (Table 5.1). Per isolate five crystallizing dishes were used. Thallus branches were exposed to high-light (10x E_k) for 120 min. Then, they were allowed to recover from the high-light treatment under dim light (approximately 3 µmol photons m^{-2} s^{-1}) for 120 min and finally over night (approximately for 16 h). High-light was provided by halogen lamps (400 W) and dim light by daylight fluorescence tubes (36 W). Experimental irradiances were measured with a LI-190 cosine corrected quantum sensor (LiCor, Lincoln, NB, USA) connected to a LI-189 radiometer (LiCor, Lincoln, NB, USA). Temperature-control was achieved by a cryostat (Model 1160S, VWR International GmbH, Darmstadt, Germany).

Measurements of F_v/F_m were carried out at the beginning of the experiment, after 15, 30, 60 and 120 min of high-light exposure as well as after 15, 30, 60 and 120 min and over-night recovery. In addition, at the beginning of the high-light exposure, five replicates per isolate were selected for the determination of the status of Mast-ex, Mast-ov and Chon-ov to assess the ecophysiological algal status in the field (for details see below).

Ecophysiological analyses

To determine differences in the ecophysiological status and potential adaptive traits of the isolates Mast-ex, Mast-ov and Chon-ov in the field, the following response variables were measured at the beginning of the high-light stress experiment: pigment concentrations (chlorophyll, carotenoids and phycobilins), antioxidant activity, total lipid content and fatty acid composition. For the different ecophysiological analyses, thallus branches were pooled to form a replicate of approximately 500 milligram fresh weight. This algal material was carefully blotted dry with paper towels, shock frozen in liquid nitrogen and stored at -80°C until further processing.

Pigments and phycobilins

Pigment determination was performed by reversed phase high performance liquid chromatography (HPLC). The algal material was lyophilized for 24 h and pulverized at 4 m s^{-1}

for 20 s in a high-speed benchtop homogenizer (FastPrep®-24; MP Biomedicals, Solon, OH, USA). Pigments from the algal material (approximately 125 mg dry weight) were extracted in 1 ml of ice-cold 90% acetone (Merck, Darmstadt, Germany) for 24 h at -20°C in the dark. After centrifugation (5 min, 4°C, 13000 *g*) and filtration through a 45 µm nylon syringe filter (Nalgene®, Nalge Nunc International, Rochester, NY, USA), HPLC analysis was performed on a LaChromElite® system equipped with a chilled autosampler L-2200 and a DAD detector L-2450 (VWR-Hitachi International GmbH, Darmstadt, Germany). A Spherisorb® ODS-2 column (25 cm x 4.6 mm, 5 µm particle size; Waters, Milford, MA, USA) with a LiChropher® 100-RP-18 guard cartridge was used for the separation of pigments, applying a gradient according to Wright et al. (1991). Peaks were detected at 440 nm and identified as well as quantified by co-chromatography with standards for chlorophyll *a* (Chl *a*), β-carotene and lutein (DHI Lab Products, Hørsholm, Denmark) using the software EZChrom Elite ver. 3.1.3. (Agilent Technologies, Santa Clara, CA, USA). Pigment concentrations were expressed as milligrams per milligram Chl *a* (with exception of Chl *a*, which was given as micrograms per milligram dry weight).

Phycobilin concentrations were determined following the method according to Beer and Eshel (1985) with slight modifications. The algal material was lyophilized and pulverized as described above. Phycobilins from the algal material (approximately 80 mg dry weight) were extracted in 1 ml 0.1M phosphate buffer, pH 6.8. After centrifugation (20 min, 10000 *g*), the absorbance of the supernatant was measured at 455 nm, 564 nm, 592 nm, 618 nm and 645 nm using a spectrophotometer (UV-2401PC; Shimadzu, Duisburg, Germany). Concentrations of phycoerythrin (E) and phycocyanin (C) in mg ml^{-1} were calculated from the absorbance (A) at the respective wavelengths as follows:

$E = [(A_{564} – A_{592}) – (A_{455} – A_{592})\ 0.20]\ 0.12$

$C = [(A_{618} – A_{645}) – (A_{592} – A_{645})\ 0.51]\ 0.15$

Phycobilin concentrations were expressed as milligrams per milligram Chl *a*.

Antioxidant activity

The antioxidant activity was measured by the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich, Seelze, Germany) scavenging method according to Cruces et al. (2012) with slight modifications. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich, Seelze, Germany) was used as a standard. A 150 µM DPPH* stock solution was prepared in ethanol (Merck, Darmstadt, Germany). The algal material was lyophilized and pulverized as described above. Antioxidants from the algal material (approximately 50 mg dry weight) were extracted in 1 ml of 70% acetone (Merck, Darmstadt, Germany) for 24 h at 4°C while shaking in the dark. Afterwards, 22 µl of the supernatant and 200 µl of the DPPH* stock solution were directly mixed in a 96-well microplate. After 15 min,

the absorbance was measured at 520 nm using a microplate reader (FLUOstar OPTIMA; BMG Labtech GmbH, Ortenberg, Germany). The antioxidant activity was estimated from triplicate subsamples, from which a mean was calculated, and expressed as milligram Trolox equivalent (TE) per milligram Chl *a*.

Total lipid content and fatty acid composition

The algal material was lyophilized for 48 h and pulverized at 1500 rpm for 1 min with liquid nitrogen in a homogenizer (Mikro-Dismembrator, Typ U; B. Braun Biotech International GmbH, Melsungen, Germany). Total lipids were extracted in dichloromethane:methanol (2:1 per volume) following the methods described by Folch et al. (1957) and Bligh and Dyer (1959). Extracts were mixed and ultrasonicated and total lipid contents were determined gravimetrically after Hagen (2000). For the analysis of fatty acid composition, aliquots of the algal extracts were taken and converted to their methyl ester derivatives (FAMEs) by transesterification with methanol (Merck, Darmstadt, Germany) containing 3% concentrated sulphuric acid (Merck, Darmstadt, Germany) for 4 h at 80°C. After extracting the FAMEs three times with hexane (Merck, Darmstadt, Germany), their composition was analyzed using a HP 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a DB-FFAP column (60 m length, 0.25 mm inner diameter, 0.25 µm film thickness; Agilent Technologies, Waldbronn, Germany) operated with temperature programming essentially after Kattner and Fricke (1986). FAMEs were identified by comparing their retention times with those derived from standards of known composition. Individual fatty acids were calculated as mass percentage of the total fatty acid content and grouped according to their degree of saturation and their chain length, with shorter-chain fatty acids being defined as fatty acids with 14 and 16 carbon atoms (C14 and C16, respectively) and longer-chain fatty acids being defined as fatty acids with 18 and 20 carbon atoms (C18 and C20, respectively).

Statistical analysis

To test for differences in the ecophysiological algal status (pigments, phycobilins, antioxidants, lipids and fatty acids) related to the factors isolate (Mast-ex, Mast-ov and Chon-ov) and sampling event (Oct 2011; Mar, May and Aug 2012), two-factorial analyses of variance (two-way ANOVA) were carried out. When the ANOVA revealed significant differences for main effects and/or the interaction, Fisher's least significant difference (LSD) procedure was applied, respectively. Prior to all statistical analyses, percentage data were arcsine-transformed. Further, all data were tested for normality and homogeneity of variances, using Kolmogorov-Smirnov's test and Levene's test, respectively. The software PASW Statistics 18 (SPSS; Armonk, NY, USA) was used for statistical analyses. Critical significance levels of 5% were applied.

RESULTS

Short-term responses in maximum quantum yield (F_v/F_m) of isolates to high-light stress

Ecophysiological changes during the high-light stress experiment in the maximum quantum yield (F_v/F_m) were calculated as percentage of initial values to enable a better comparability between the three isolates. The changes in F_v/F_m of the algal isolates with respect to high-light stress and subsequent recovery differed between the various sampling events (Figure 5.1). In Mar and May 2012, the responses of the algal isolates were very similar (Figure 5.1b and c), whereas they showed clear isolate-specific differences in Oct 2011 and Aug 2012 (Figure 5.1a and d). In Mar 2012, there was almost no decrease in F_v/F_m after 120 min of high-light exposure in the *M. stellatus* and *C. crispus* isolates and the values returned quickly to the initial values during the recovery period (Figure 5.1b). In May 2012, F_v/F_m declined to approximately 60% of initial values in all three isolates after the high-light stress (120 min; Figure 5.1c) and was able to increase again to above 90% of the initial values after over-night recovery (data not shown). In Oct 2011, the decrease of F_v/F_m during the high-light stress was strongest and fastest in Chon-ov (to 70% and 50% of initial values after 15 min and 120 min, respectively), followed by Mast-ov (to 95% and 60% of initial values after 15 min and 120 min, respectively) and Mast-ex (almost no decrease and to 75% of initial values after 15 min and 120 min, respectively; Figure 5.1a). During the recovery phase, the maximum quantum yields of Mast-ex and Mast-ov recovered quickly from high-light stress (Figure 5.1a), whereas F_v/F_m of Chon-ov only reached 75% of the initial values even after over-night recovery (data not shown). In Aug 2012, the response of F_v/F_m to high-light exposure and following recovery was similar compared to that observed in Oct 2011 (Figure 5.1d). However, all three algal isolates were able to reach 90-100% of their initial F_v/F_m values after the recovery period over-night (data not shown).

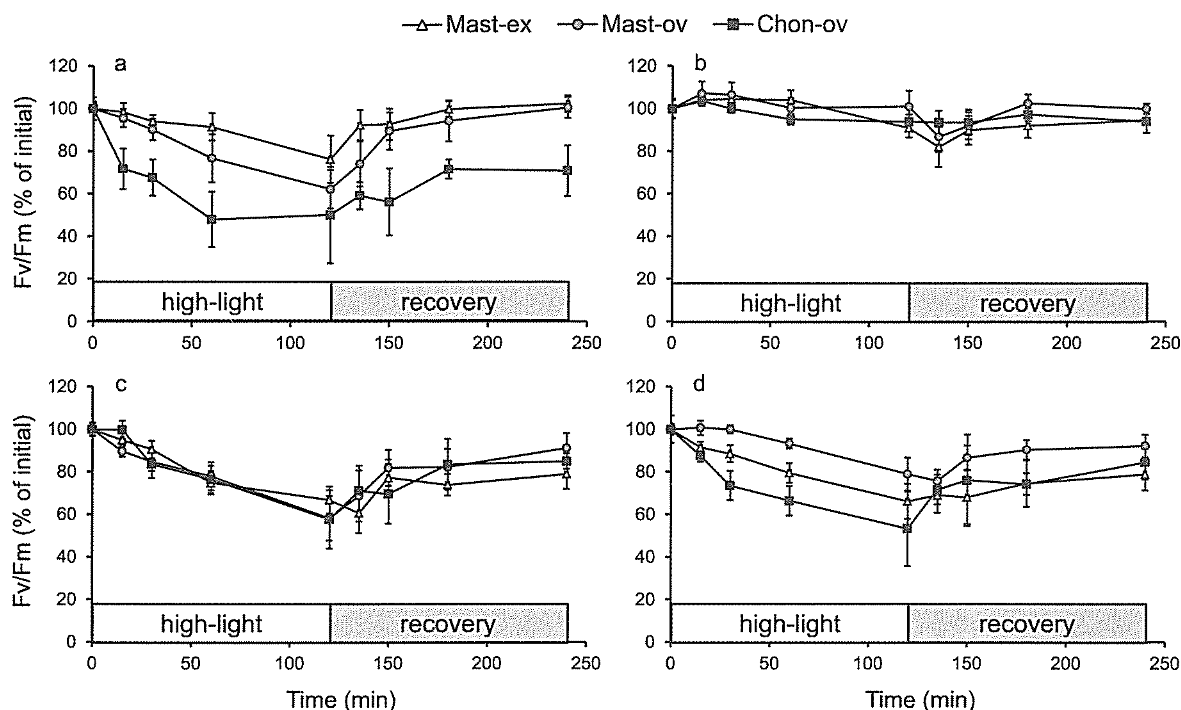

Figure 5.1: Maximum quantum yields (F_v/F_m; % of initial) of thallus branches of *Mastocarpus stellatus* and *Chondrus crispus* during exposure to high light (10x E_k; 0 to 120 min) and recovery from the high-light treatment under dim light (approximately 3 µmol photons m^{-2} s^{-1}; 120 to 240 min) in **(a)** October 2011, **(b)** March 2012, **(c)** May 2012 and **(d)** August 2012. Measurements of F_v/F_m were carried out at the beginning of the experiment (0 min), after 15, 30, 60 and 120 min of high-light exposure as well as after 15, 30, 60 and 120 min of recovery. To allow a better comparability between the three algal isolates (Mast-ex, Mast-ov, Chon-ov), F_v/F_m was calculated as percentage of initial values. Data points are means±95% confidence intervals (n=5). Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal.

Ecophysiological status of isolates

Over the consecutive sampling events, changes in the ecophysiological status of the red algal isolates have been detected. For a better comparability between the three isolates, Chl *a* was used as denominator for the calculation of pigment concentrations and antioxidant activity. The Chl *a* concentration was highest in Chon-ov, significantly lower in Mast-ex and again significantly lower in Mast-ov (Tables 5.2 and 5.3). In contrast, the β-carotene and lutein concentrations did not show consistent isolate-specific differences between the four sampling events (Tables 5.2 and 5.3). The concentrations of the phycobilins phycoerythrin and phycocyanin did also not differ significantly with respect to the factors isolate and sampling event (Tables 5.2 and 5.3). For the majority of the sampling events, the antioxidant activity was significantly higher in the two *Mastocarpus stellatus* isolates than in *Chondrus crispus* (Figure 5.2 and Table 5.3). In contrast, the total lipid content was significantly lower in Mast-ex and Mast-ov compared to Chon-ov (Tables 5.2 and 5.3).

The sum of saturated fatty acids (SFA) and the sum of polyunsaturated fatty acids (PUFA) did not differ significantly between the three algal isolates within each sampling event (Tables 5.2 and 5.3). Contrarily, the sum of monounsaturated fatty acids (MUFA) exhibited significant isolate-specific differences, with highest contents in Mast-ov, followed by those in Mast-ex and lowest contents in Chon-ov (Tables 5.2 and 5.3). Following the differences in the various saturation states of fatty acids, the saturated/unsaturated fatty acid ratio (SFA/UFA) showed no consistent pattern with respect to algal isolate over the course of one year (Tables 5.2 and 5.3). However, the shorter-chain/longer-chain fatty acid ratio (C14+C16/C18+C20) was significantly higher in Mast-ex and Mast-ov compared to Chon-ov within each of the four sampling events (Tables 5.2 and 5.3). Totally, nine different fatty acids were identified in the algal isolates (exemplarily shown for Aug 2012, Table S5.1). The saturated fatty acid 16:0 and the three unsaturated fatty acids 18:1(n-9), 20:4(n-6) and 20:5(n-3) made up almost 90% of the total fatty acids in the algae. Other fatty acids, detected only in minor amounts, were 14:0, 16:1(n-7), 18:0, 18:1(n-7) and 18:2(n-6). Significant isolate-specific differences were found for four single fatty acids. Within each sampling event, both *M. stellatus* isolates represented higher amounts of 16:1(n-7) ($p<0.001$) and lower amounts of 18:0 ($p=0.021$), 18:2(n-6) ($p<0.001$) as well as 20:4(n-6) ($p<0.001$) compared to *C. crispus* (Table S5.1).

Table 5.2: Chlorophyll *a* (Chl *a*) concentration, ratios of pigments to chlorophyll *a* (β-carotene/Chl *a*, lutein/Chl *a*, phycoerythrin/Chl *a*, phycocyanin/Chl *a*), total lipid content, saturation states of fatty acids (sum of saturated fatty acids (SFA), sum of monounsaturated fatty acids (MUFA), sum of polyunsaturated fatty acids (PUFA), saturated/unsaturated fatty acid ratio (SFA/UFA)) and shorter-chain/longer-chain fatty acid ratio (C14+C16/C18+C20) of initial thallus branches of *Mastocarpus stellatus* and *Chondrus crispus* collected on four sampling events (Oct 2011; Mar, May and Aug 2012). Table shows means±SEM (n=5). Dark circles indicate isolate-specific (Mast-ex, Mast-ov and Chon-ov) differences within all four sampling events that are significant at p<0.05 (significant isolate effect of two-way ANOVA followed by a Fisher's LSD test). Different letters (a and b) indicate significant differences among algal isolates within one of the four sampling events (significant interaction Isolate x Sampling event of two-way ANOVA followed by a Fisher's LSD test, p<0.05).

Response variables of initial thallus branches	**Sampling event**			
	Oct 2011	**Mar 2012**	**May 2012**	**Aug 2012**
Chl *a* (µg mg^{-1} dry weight)•				
Mast-ex	0.31±0.01	0.26±0.01	0.30±0.01	0.31±0.02
Mast-ov	0.23±0.02	0.26±0.01	0.28±0.01	0.22±0.01
Chon-ov	0.32±0.03	0.33±0.01	0.40±0.04	0.35±0.03
β-carotene/Chl *a*				
Mast-ex	0.11±0[a]	0.11±0[a]	0.12±0[a]	0.11±0[b]
Mast-ov	0.11±0[a]	0.11±0[a]	0.12±0[a]	0.11±0[b]
Chon-ov	0.11±0[a]	0.11±0[a]	0.11±0[b]	0.12±0[a]
Lutein/Chl *a*				
Mast-ex	0.21±0.01[a]	0.23±0[a]	0.25±0.01[a]	0.23±0[a]
Mast-ov	0.19±0.01[b]	0.20±0.01[b]	0.24±0.01[a]	0.23±0[a]
Chon-ov	0.17±0.01[b]	0.19±0.01[b]	0.18±0.01[b]	0.20±0.01[b]

Table 5.2: Continued.

Response variables of initial	Sampling event			
thallus branches	**Oct 2011**	**Mar 2012**	**May 2012**	**Aug 2012**
Phycoerythrin/Chl *a*				
Mast-ex	1.48±0.29	1.72±0.12	2.05±0.23	2.11±0.72
Mast-ov	1.70±0.30	2.82±0.95	1.48±0.28	2.01±0.78
Chon-ov	2.24±0.33	1.76±0.36	1.47±0.19	2.10±0.47
Phycocyanin/Chl *a*				
Mast-ex	0.27±0.05	0.26±0.02	0.30±0.04	0.37±0.10
Mast-ov	0.30±0.04	0.41±0.12	0.24±0.04	0.39±0.15
Chon-ov	0.45±0.08	0.47±0.09	0.32±0.03	0.50±0.11
Total lipids (% of dry weight)				
Mast-ex	1.4±0.2[b]	1.2±0.1[b]	1.2±0.1[b]	1.6±0.2[a]
Mast-ov	1.1±0.1[b]	1.0±0.1[b]	1.2±0[b]	1.1±0[b]
Chon-ov	1.7±0.1[a]	1.9±0.1[a]	1.7±0[a]	1.3±0.1[b]
SFA (mass % of total fatty acids)				
Mast-ex	35.6±0.7	30.4±0.2	32.0±0.2	37.6±0.7
Mast-ov	34.4±0.3	30.6±0.2	32.1±0.1	37.1±0.8
Chon-ov	36.3±1.4	30.4±0.4	32.0±0.3	34.4±0.4
MUFA (mass % of total fatty acids)•				
Mast-ex	10.4±1.5	13.2±0.2	11.3±0.1	9.5±1.3
Mast-ov	13.7±0.7	13.5±0.1	10.6±0.1	10.4±0.2
Chon-ov	9.7±0.4	8.6±0.3	9.9±0.2	9.4±0.2
PUFA (mass % of total fatty acids)				
Mast-ex	54.0±0.9	56.4±0.1	56.7±0.2	53.0±0.7
Mast-ov	52.0±0.4	55.9±0.2	57.3±0.2	52.5±1.0
Chon-ov	54.1±1.2	61.0±0.4	58.1±0.3	56.2±0.2
SFA/UFA				
Mast-ex	0.55±0.02[ab]	0.44±0[a]	0.47±0[a]	0.60±0.02[a]
Mast-ov	0.52±0.01[b]	0.44±0[a]	0.47±0[a]	0.59±0.02[a]
Chon-ov	0.57±0.03[a]	0.44±0.01[a]	0.47±0.01[a]	0.52±0.01[b]
C14+C16/C18+C20•				
Mast-ex	0.58±0.02	0.46±0	0.49±0	0.62±0.02
Mast-ov	0.56±0.01	0.47±0	0.49±0	0.60±0.02
Chon-ov	0.52±0.01	0.42±0.01	0.46±0.01	0.50±0.01

Aug, August; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; Mar, March; Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Oct, October; SEM, standard error of the mean

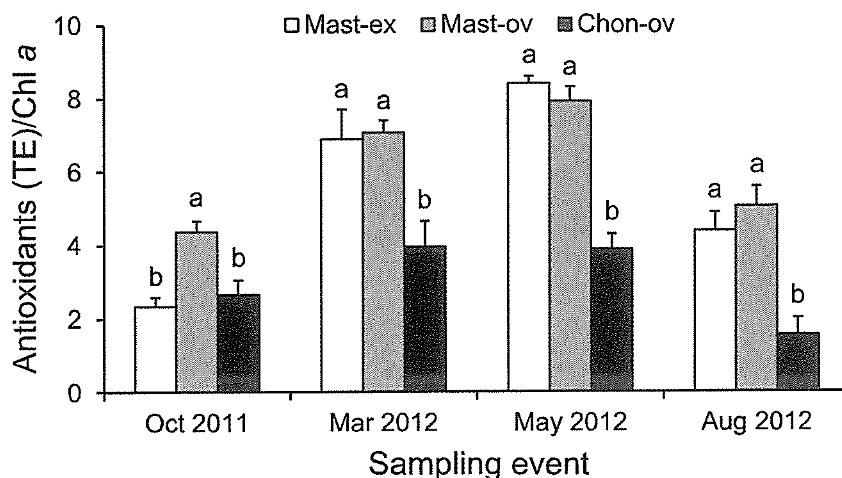

Figure 5.2: Molar ratio of antioxidants (TE) to chlorophyll *a* (antioxidants(TE)/Chl *a*) of initial thallus branches of *Mastocarpus stellatus* and *Chondrus crispus* collected on four sampling events (Oct 2011; Mar, May and Aug 2012). Bars are means±SEM (n=5). Different letters (a and b) indicate significant differences among algal isolates within one of the four sampling events (significant interaction Isolate x Sampling event of two-way ANOVA followed by a Fisher's LSD test, $p<0.05$). Aug, August; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; Mar, March; Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Oct, October; SEM, standard error of the mean.

Table 5.3: Statistical evaluation of chlorophyll *a* (Chl *a*) concentration, ratios of pigments (β-carotene/Chl *a*, lutein/Chl *a*, phycoerythrin/Chl *a*, phycocyanin/Chl *a*) and antioxidant (antioxidants (TE)/Chl *a*), total lipid content, saturation states of fatty acids (sum of saturated fatty acids (SFA), sum of monounsaturated fatty acids (MUFA), sum of polyunsaturated fatty acids (PUFA), saturated/unsaturated fatty acid ratio (SFA/UFA)) and shorter-chain/longer-chain fatty acid ratio (C14+C16/C18+C20) of initial thallus branches of *Mastocarpus stellatus* and *Chondrus crispus*, using two-factorial analysis of variance, with the factors sampling event (Oct 2011; Mar, May and Aug 2012) and isolate (Mast-ex, Mast-ov and Chon-ov) and their interaction. p-values in bold highlight significant differences at $p<0.05$.

					Chl *a*					**β-carotene/Chl *a***
Source of variation	**SS**	**df**	**MS**	**F-ratio**	**p-value**	**SS**	**df**	**MS**	**F-ratio**	**p-value**
Isolate	0.115	2	0.057	32.101	**<0.001**	3.0×10^{-5}	2	1.5×10^{-5}	0.545	0.583
Sampling event	0.016	3	0.005	2.985	**0.040**	2.2×10^{-4}	3	7.3×10^{-5}	2.646	0.060
Isolate x Sampling event	0.019	6	0.003	1.786	0.122	0.001	6	1.3×10^{-4}	4.586	**0.001**
Error	0.086	48	0.002			0.001	48	2.8×10^{-5}		

					Lutein/Chl *a*					**Phycoerythrin/Chl *a***
Source of variation	**SS**	**df**	**MS**	**F-ratio**	**p-value**	**SS**	**df**	**MS**	**F-ratio**	**p-value**
Isolate	0.021	2	0.011	44.316	**<0.001**	0.273	2	0.137	0.115	0.891
Sampling event	0.011	3	0.004	14.937	**<0.001**	1.988	3	0.663	0.560	0.644
Isolate x Sampling event	0.004	6	0.001	2.731	**0.023**	6.260	6	1.043	0.882	0.516
Error	0.012	48	2.4×10^{-4}			56.805	48	1.183		

					Phycocyanin/Chl *a*					**Antioxidants (TE)/Chl *a***
Source of variation	**SS**	**df**	**MS**	**F-ratio**	**p-value**	**SS**	**df**	**MS**	**F-ratio**	**p-value**
Isolate	0.188	2	0.094	2.790	0.071	106.940	2	53.470	49.380	**<0.001**
Sampling event	0.142	3	0.047	1.402	0.254	137.450	3	45.817	42.312	**<0.001**
Isolate x Sampling event	0.081	6	0.014	0.401	0.875	30.891	6	5.149	4.755	**0.001**
Error	1.618	48	0.034			51.976	48	1.083		

					Total lipids					**SFA**
Source of variation	**SS**	**df**	**MS**	**F-ratio**	**p-value**	**SS**	**df**	**MS**	**F-ratio**	**p-value**
Isolate	0.001	2	3.4×10^{-4}	44.667	**<0.001**	0.006	2	0.003	1.428	0.250
Sampling event	1.8×10^{-5}	3	6.1×10^{-6}	0.815	0.492	0.023	3	0.008	3.468	**0.023**
Isolate x Sampling event	3.2×10^{-4}	6	5.3×10^{-5}	7.037	**<0.001**	0.028	6	0.005	2.071	0.074
Error	3.6×10^{-4}	48	7.5×10^{-6}			0.108	48	0.002		

					MUFA					**PUFA**
Source of variation	**SS**	**df**	**MS**	**F-ratio**	**p-value**	**SS**	**df**	**MS**	**F-ratio**	**p-value**
Isolate	0.011	2	0.006	16.058	**<0.001**	0.001	2	3.4×10^{-4}	0.055	0.947
Sampling event	0.005	3	0.002	5.250	**0.003**	0.079	3	0.026	4.332	**0.009**
Isolate x Sampling event	0.005	6	0.001	2.277	0.052	0.036	6	0.006	0.987	0.445
Error	0.017	48	3.8×10^{-4}			0.293	48	0.006		

Table 5.3: Continued.

	SFA/UFA					C14+C16/C18+C20				
Source of variation	**SS**	**df**	**MS**	**F-ratio**	**p-value**	**SS**	**df**	**MS**	**F-ratio**	**p-value**
Isolate	0.002	2	0.001	0.801	0.455	3.4×10^{-5}	2	1.7×10^{-4}	4.073	**0.023**
Sampling event	0.177	3	0.059	55.952	**<0.001**	0.001	3	3.9×10^{-4}	93.871	**<0.001**
Isolate x Sampling event	0.018	6	0.003	2.857	**0.019**	3.9×10^{-5}	6	6.4×10^{-6}	1.550	0.183
Error	0.050	47	0.001			2.0×10^{-4}	47	4.1×10^{-6}		

Aug, August; Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; Mar, March; Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; Oct, October

DISCUSSION

Overall, the results of the present study revealed that *Mastocarpus stellatus* and *Chondrus crispus* differ in their high-light tolerance, with *M. stellatus* generally being less sensitive to this stress factor. Further, the algal isolates exhibited significant differences in a number of ecophysiological characteristics (antioxidants, pigments, total lipids, fatty acid composition) tested, which seem to be species-specific rather than habitat-specific.

During the high-light stress experiments, we observed the typical pattern of photoinhibition (decrease of F_v/F_m) and subsequent recovery after the end of the stress exposure, with the completeness of recovery depending on the algal isolate and season (e.g., Dring et al. 1996, Bischof et al. 2000). In line with our results, previous studies found that the sensitivity of photoinhibition towards abiotic stress differs with the vertical position of red algae on the shore (Dring et al. 1996, Sagert et al. 1997, Bischof et al. 2000). Dring et al. (1996) assumed that the sensitivity to UV radiation of red algae occurring around the island of Helgoland varies amongst others with growth depth of algae. Here, the rate of the initial decline of F_v/F_m during UV exposure was greatest and the extent of recovery was less pronounced in deep subtidal species, like *Delesseria sanguinea* and *Plocamium cartilagineum*, than in intertidal or shallow subtidal species. Sagert et al. (1997) observed a similar response in *C. crispus* from various growth depths (3.5 to 8.5 m below high tide level) on the western Atlantic coast of Brittany, France, when those plants were exposed to irradiance of PAR and UV. The latter finding might indicate an acclimation to the irradiance at the respective growth depths of this species.

The intensity of solar radiation not only differs with respect to vertical zonation on the shore, but also deviated strongly with respect to season (Dring et al. 2001), so that we expected differences in the responses of F_v/F_m of the algal isolates to the high-light stress between the four sampling events. In particular, isolate-specific differences were thought to be distinct in the months with higher levels of solar radiation (April to September with an overall monthly mean of 1600 µmol photons m^{-2} s^{-1}) and should be lower in those with less solar irradiance (October to March with an overall monthly mean of 570 µmol photons m^{-2} s^{-1}; Dring et al. 2001). Actually, we found clear isolate-specific differences in Aug 2012 and Oct 2011, whereas in Mar and May 2012 the responses of the algal isolates were very similar. These findings did not correlate very well with the seasonal pattern of solar radiation. However, the sensitivity of *C. crispus* to the high-light stress seemed to be influenced by the prevailing water temperature. *Chondrus crispus* is able to grow over a wide temperature range from 5 to 20°C (Kübler and Davison 1993), with maximal growth and photosynthetic rates at 15°C (van den Hoek 1982, Pettitt et al. 1989). Further, thermal acclimation to growth temperature was found to exist in this algal species, so that individuals acclimated to summer seawater temperatures (20°C) can tolerate brief exposures to extremely high temperatures

better than those acclimated to winter seawater temperatures (5°C; Kübler and Davison 1993). Nevertheless, our findings indicated that the high-light tolerance of *C. crispus* is less pronounced than that of *M. stellatus* in late summer and autumn (Aug 2012 and Oct 2011 with water temperatures of 16°C and 14°C, respectively) at higher water temperatures as compared to the other sampling events (May and Mar 2012 with water temperatures of 8°C and 4°C, respectively). This is consistent with findings for *C. crispus* from Maine, USA by Kübler and Davison (1993), showing that light has a profound effect on the response of this species to high temperature. The photosynthesis of algae, acclimated to a temperature of 20°C, was not inhibited by the exposure to 30°C at moderate light levels (70-100 µmol photons m^{-2} s^{-1}), but inhibition did occur when those algae were exposed to high light levels (600 µmol photons m^{-2} s^{-1}). Since air temperature during tidal emersion may be 10-20°C higher (or lower) than water temperature (Davison and Pearson 1996), temperatures around 30°C can easily be reached on Helgoland during summer and autumn.

Differences in ecophysiological characteristics might contribute to the generally higher stress tolerance of *M. stellatus* compared to *C. crispus* (Davison et al. 1989, Dudgeon et al. 1989, 1995, Bischof et al. 2000). We were able to show that, regardless of the position on the shore, *M. stellatus* possessed a higher antioxidant activity than *C. crispus* during the majority of the sampling events. This is in line with a study by Collén and Davison (1999), who reported about a generally higher efficiency of the reactive oxygen metabolism and resistance to oxidative stress in *M. stellatus* (higher levels of ascorbate and β-carotene and higher activities of catalase and glutathione reductase) in comparison to *C. crispus*. However, this generality could not be confirmed in another investigation on the seasonal acclimatization of antioxidants in the same two red algal species (Lohrmann et al. 2004). Here, *M. stellatus* only had higher ascorbate contents, whereas the activities of the enzymes superoxide dismutase and ascorbate peroxidase were greater in *C. crispus*. We suggest, that the higher antioxidant activity might allow *M. stellatus* to live at higher positions on the shore. Algal organisms living in those habitats are in particular exposed to several environmental stress factors, which are known to stimulate the formation of ROS. Thus, an effective defense system against ROS is necessary for their survival (Mallick and Mohn 2000). Generally higher antioxidant activities at colder water temperatures (Mar 12 and May 12) in the three algal isolates might also emphasize the importance of this defense system during coldness. Those cold-induced increases in antioxidants are thought to compensate for the effect of lower temperatures on their activities and for the generation of ROS, which is particularly high when chilling and freezing events occur (Lohrmann et al. 2004).

As for the antioxidants, the red algal isolates also differed in their Chl *a* contents, with highest contents in *C. crispus*. This is part of a well-known photoacclimatory adjustment

found in algal species from different shore levels. By increasing the concentration of chlorophyll, the utilization of solar radiation becomes more efficient for *C. crispus* in low light environments at greater water depths. Vice versa, excessive absorption of light is avoided in *M. stellatus* (particularly in Mast-ex) by lower chlorophyll amounts in shallower waters. Additionally, respective acclimations in antenna pigments (e.g., phycobilins), which result in further adjustments of light harvesting to various light climates, were also frequently observed (Mathieson and Norall 1975, Becker et al. 2000). Why those pigments did not show clear species- or habitat-specific differences in our study remains unresolved.

Overall, total lipid contents in *M. stellatus* and *C. crispus* were relatively low (approximately 1.5% of dry weight). This agrees with a study on five macroalgal species by Herbreteau et al. (1997), who also propose that very low total lipid levels appear to be characteristic for plants living in marine environments. Species-specific differences in total lipids were found during most of the sampling events, usually with higher contents in *C. crispus* than in *M. stellates*. Previous studies detected greater amounts of total lipids in individuals of the red macroalgae *Grateloupia turuturu* (Khotimchenko 2002) and *Tichocarpus crinitus* (Khotimchenko and Yakovleva 2005) as well as of the red microalga *Porphyridium cruentum* (Klyachko-Gurvich et al. 1999) growing at low solar radiation compared to those being exposed to high light intensities. Thus, differences in total lipids in *M. stellatus* and *C. crispus* might also be due to variations in the light climates along the vertical gradient on the shore, with decreasing levels of solar irradiance with depth.

Major fatty acids found in the three algal isolates were 16:0, 18:1(n-9), 20:4(n-6) and 20:5(n-3), which agrees with the fatty acid compositions of many other red algae (e.g., Jamieson and Reid 1972, Pettitt et al. 1989, Fleurence et al. 1994, Tasende 2000, Khotimchenko et al. 2002). It is already known that the fatty acid composition of *C. crispus* varies with respect to the phase of the life cycle (Tasende 2000) and with respect to environmental conditions, such as light (Pettitt and Harwood 1989) and temperature (Pettitt et al. 1989). However, to our knowledge, a comparative study of the fatty acid compositions between *M. stellatus* and *C. crispus* was not yet conducted. We found higher contents of MUFA in the two isolates of *M. stellatus* compared to that of *C. crispus*. Further, we detected species-specific differences in the C14+C16/C18+C20 ratio, with higher values in *M. stellatus*. This means that *M. stellatus* exhibited a higher degree of unsaturation and more shorter-chain fatty acids than *C. crispus*, with both characteristics resulting in a higher fluidity of biomembranes (Buchanan et al. 2000). Previous studies highlighted differences in fatty acid composition in green, brown and red macroalgae with respect to growth depth on the shore, with a higher degree of unsaturation in shallower compared to deeper waters (Ito and Tsuchiya 1977, Becker et al. 2010). Apparently, in some red algae, fatty acid unsaturation is stimulated by an increase in light intensity (Pettitt and Harwood 1989, Levy et al. 1992).

Since those high light conditions exist in shallower waters around Helgoland, we propose that they might contribute to the rise in fatty acid unsaturation in this habitat. Shallower waters are characterized by extremely variable environmental conditions, including fluctuations in PAR and UV radiation as well as temperature, which is probably quite stressful for algae living there (Kübler and Davison 1993). Under these conditions, the formation of ROS is known to increase, which in turn might promote the degradation of the D1 reaction center protein of PSII. To maintain a high photosynthetic capacity, degraded proteins must be removed and *de novo* synthesized proteins must be re-integrated into the reaction centers (i.e., D1 protein turnover). This process takes place via lateral diffusion through the thylakoid membranes and is facilitated within more fluid membranes (Becker et al. 2010 and references therein). Further, electron and ion transport can be easier performed through a membrane with a higher fluidity (Klyachko-Gurvich et al. 1999). Therefore, higher levels of fatty acid desaturation might help *M. stellatus* to maintain biomembranes, especially thylakoid membranes containing the photosynthetic apparatus, operative in a wide range of environmental conditions in shallower water depths.

CONCLUSIONS

Our study showed that *Mastocarpus stellatus* has a higher tolerance towards high-light stress than *Chondrus crispus*. New insights were provided into possible adaptive mechanisms of stress tolerance, indicated by differences in several ecophysiological characteristics (antioxidants, pigments, total lipids, fatty acid composition) between the algal isolates. In this regard, the two *M. stellatus* isolates from two shore levels differed from *C. crispus* with respect to the antioxidants, total lipids and C14+C16/C18+C20. These differences appear to be genetically determined and hence species-specific, since they are not masked by responses to various environmental settings along the depth gradient (habitat-specific differences). Such differences in ecophysiology might enhance the tolerance to different abiotic stress factors, but might also allow rapid recovery from this stress in *M. stellatus*. It might explain why this species is more competitive in the highly variable upper intertidal compared to *C. crispus*. Since we assumed that high-light tolerance in *C. crispus* is negatively affected by higher water temperatures, the competition between both species might be modulated in the future under rising mean annual sea surface temperature around the island of Helgoland (Wiltshire et al. 2009). To predict competitive interactions between the two species, future studies should determine the tolerance to high-light stress at various temperature levels. Further, more detailed studies should focus on changes in fatty acid composition within different lipid classes of the two species, which would allow identification of which specific membranes (e.g., thylakoid membranes) are primarily affected by the observed differences in fatty acids.

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SUPPORTING INFORMATION

Table S5.1: Fatty acid composition of initial thallus branches of *Mastocarpus stellatus* and *Chondrus crispus* collected in August 2012. Table shows means±SEM (n=5).

Fatty acid	Isolate		
	Mast-ex	Mast-ov	Chon-ov
14:0	3.4±0.1	3.2±0.1	3.4±0.1
16:0	32.0±0.7	31.2±0.6	29.5±0.6
16:1(n-7)	2.0±0.1	1.8±0.1	0.4±0.0
18:0	1.3±0.2	1.4±0.2	1.3±0.1
18:1(n-7)	2.0±0.0	1.4±0.1	0.9±0.0
18:1(n-9)	5.3±1.3	6.9±0.2	8.0±0.2
18:2(n-6)	1.0±0.0	1.0±0.0	1.6±0.6
20:4(n-6)	25.0±0.4	23.7±0.4	25.9±0.4
20:5(n-3)	25.8±0.4	26.0±0.7	28.1±0.1

Chon-ov, *Chondrus crispus* from overlapping zone in deeper levels of lower intertidal; Mast-ex, *Mastocarpus stellatus* from higher levels of the lower intertidal; Mast-ov, *Mastocarpus stellatus* from overlapping zone in deeper levels of lower intertidal; SEM, standard error of the mean

6 SUMMARY OF RESULTS

In the present chapter, the most striking findings of the three studies (**Publications I-III**) included in this dissertation are summarized, dealing particularly with the significance of adjustments of membrane fatty acid composition to macroalgal photoacclimation and -protection along various gradients of light and temperature. All results are reported in full in the corresponding publications (Chapters 3-5).

6.1 Photoacclimation and -protection along a latitudinal gradient

In **Publication I**, dealing with photoacclimatory and -protective mechanisms along a latitudinal gradient of the northern-central Chilean coast, the ecophysiological characteristics of the two brown algal species *Lessonia berteroana* (distribution: 16°S-30°S) and *Lessonia spicata* (distribution: 29°S-40°S), both belonging to the *Lessonia nigrescens* complex, were determined. In doing so, algal individuals from eight different sampling locations (27°S-32°S, for details see Table 3.1), including the monospecific core zone of each species and the narrow species' overlapping zone (29°S-30°S), were compared. In general, most of the ecophysiological parameters (variable Chl *a* fluorescence of PSII, pigments and phlorotannins) tested exhibited clear species-specific differences. As these differences were predominantly found along the full latitudinal range and within the overlapping zone, the variation is considered to be irrespective of the geographic latitude. In total, 13 different fatty acids (four saturated and nine unsaturated fatty acids) were detected in the two species (for details see Table 3.2), but the total lipid content, the different fatty acid saturation states and the fatty acid composition did not differ by species or sampling location (Table 3.2). However, *L. berteroana* exhibited a higher photosynthetic capacity (ETR_{max}) and saturating irradiance (E_k; Figure 3.4) as well as greater phlorotannin (Figure 3.2) and pigment contents (Chl *a*, Chl *c*, fucoxanthin; Table 3.3) compared to *L. spicata*. Furthermore, the pool size of the xanthophyll cycle pigments (VAZ; Figure 3.3a) was larger in *L. berteroana* than in *L. spicata*, whereas the de-epoxidation state (DPS) of the xanthophyll cycle showed significant differences with respect to sampling location, with by trend higher values at lower latitudes (Figure 3.3b). Overall, the results of **Publication I** indicate that total lipids and fatty acid composition are negligible in species differentiation within the *L. nigrescens* complex along the northern-central Chilean coast.

6.2 Photoacclimation and -protection along different vertical gradients and its seasonal variability

Publication II concentrated on photoacclimatory and -protective processes of the brown macroalga *Macrocystis pyrifera* along a depth gradient of 4 m in northern-central Chile on

various temporal scales. Different ecophysiological acclimation strategies to various water depths between different sampling times (austral summer and winter, long-term acclimation) and more rapid adjustments to various depths during a 14-day transplantation experiment (short-term acclimation) were found, with the latter being further modulated by season. In total, 15 various fatty acids (five saturated and ten unsaturated fatty acids) were detected in *M. pyrifera* (detailed information in Table S4.5). Generally, the total lipid content and the saturated/unsaturated fatty acid ratio (SFA/UFA) of *M. pyrifera* tended to be higher in austral summer compared to austral winter across all water depths and independently of acclimation time (long- and short-term; Figures 4.1e-h and 4.3c and d). During long-term acclimation in austral summer, algal samples from 0.2 m water depth exhibited one and a half times lower total lipid contents and a higher degree of fatty acid saturation than those from 4 m depth (Figure 4.3c and d). Similarly, *M. pyrifera* responded to the short-term transplantation from 4 m to 0.2 m depth in austral winter with a rise in the fatty acid saturation (Figure 4.1h). Furthermore, a switch from shorter- to longer-chain fatty acids was observed (Figure 4.4). In addition to these acclimation responses, adjustments of variable Chl *a* fluorescence of PSII, pigment composition (Chl *c*, fucoxanthin), DPS, phlorotannins and antioxidants were responsible for the development of different ecophysiological algal states along the vertical gradient, with phlorotannins possessing a crucial role during long-term acclimation and antioxidants being important during short-term acclimation (for details see Tables 4.2 and 4.3 and Figures 4.1 and 4.3). In total, the findings of **Publication II** reveal that modifications in total lipids and fatty acid composition contribute to depth acclimation in *M. pyrifera* to an extent comparable to the other response variables. The adjustments with respect to lipids and fatty acids varied on different temporal scales (long- and short-term acclimation), but they seemed to occur at various seasons.

In **Publication III**, discussing photoacclimation and -protection along a vertical gradient (approximately 0.5-1 m) of the south-western coast of Helgoland, the ecophysiological status of the two red macroalgae *Mastocarpus stellatus* and *Chondrus crispus* was characterized over the course of a year (March, May, August and October). Thereby, algal individuals from various positions on the shore were compared: *M. stellatus* (isolate Mast-ex) from within the monospecific zone in higher levels of the lower intertidal as well as *M. stellatus* (isolate Mast-ov) and *C. crispus* (isolate Chon-ov) from within the species' overlapping zone in deeper levels of the lower intertidal. The three algal isolates exhibited differences in several ecophysiological characteristics (total lipids, fatty acid composition and antioxidants) tested, which seem to be species-specific rather than habitat-specific, with habitat being defined as vertical position on the shore. Those variations were further modulated by season. In total, nine different fatty acids (three saturated and six unsaturated fatty acids) were detected in the algae (Table S5.1 shows the fatty acid profiles of the algal isolates exemplarily for one

season). Generally, across all algal isolates, the SFA/UFA ratio and the shorter-chain/longer-chain fatty acid ratio (C14+C16/C18+C20) tended to be higher in months with a warmer water temperature (August and October) than in months with a colder water temperature (March and May), whereas the total lipid content did not differ between the various sampling events (Table 5.2). The two *M. stellatus* isolates exhibited lower total lipid contents, greater amounts of monounsaturated fatty acids (MUFA) and a higher C14+C16/C18+C20 ratio compared to the *C. crispus* isolate (Table 5.2). Furthermore, for the majority of the sampling events, the antioxidant activity was higher in the *M. stellatus* isolates than in *C. crispus* (Figure 5.2). The Chl *a* content, in contrast, was highest in Chon-ov, significantly lower in Mast-ex and again significantly lower in Mast-ov (Table 5.2). Overall, the results of **Publication III** indicate that species-specific differences in total lipids and fatty acid composition contribute to depth acclimation in *M. stellatus* and *C. crispus* and may account for variation in abiotic stress tolerance between the two species. These differences in lipids and fatty acids occurred predominantly irrespective of season.

7 SYNOPTIC DISCUSSION

Around the world, marine macroalgae, particularly the species inhabiting the intertidal zone, occupy highly variable habitats. They are exposed to environmental gradients on various spatial and temporal scales (e.g., Hutchins 1947, Breeman 1988, Broitman et al. 2001). Variations in abiotic conditions bear the potential to cause harmful oxidative stress, if they exceed the upper or lower threshold values of tolerance (Davison and Pearson 1996, Collén and Davison 1999, Mallick and Mohn 2000, Lohrmann et al. 2004). Consequently, to prevent severe chronic photoinhibition or photodamage, macroalgae rely on efficient photoacclimatory and -protective mechanisms, which implicate a high ecophysiological plasticity (Davison and Pearson 1996 and references therein). Several aspects of macroalgal photoacclimation and -protection, including photosynthetic pigments, the xanthophyll cycle, phlorotannins and antioxidants, have already been investigated (e.g., Yamamoto et al. 1962, Demmig-Adams 1990, Pfündel and Bilger 1994, Pavia et al. 1997, Asada 1999, Mallick and Mohn 2000, Schoenwaelder 2002, Koivikko et al. 2005, Gómez and Huovinen 2010, Cruces et al. 2012). Contrarily, the role of adjustments in membrane lipid composition in photoacclimation and -protection is widely understudied, although it is known that the maintenance of the thylakoid membrane integrity under variable environmental conditions is crucial for the physiological performance of macroalgae (Ohad et al. 1984, Barber and Andersson 1992, Aro et al. 1993, Becker et al. 2010). The few available studies on this aspect of macroalgal ecophysiology, indeed, showed that the fatty acid composition is influenced by changes in light and/or temperature conditions, but they did not reveal consistent responses (e.g., Al-Hasan et al. 1991, Somerville and Browse 1991, Dawes et al. 1993, Floreto et al. 1993, Floreto and Teshima 1998, Khotimchenko and Yakovleva 2005).

The major aim of the present thesis was to provide further insights into the role of membrane fatty acid composition in macroalgal photoacclimation and -protection along different spatial (latitudinal and vertical) and temporal (seasonal) gradients of light and temperature. For these studies, various brown (*Macrocystis pyrifera*, *Lessonia berteroana* and *Lessonia spicata*) and red (*Mastocarpus stellatus* and *Chondrus crispus*) macroalgal species were exposed to the different abiotic conditions prevailing along these gradients. Thereby, irradiance and water temperature was found to decrease with increasing latitude on the geographic scale (**Publication I**) as well as with increasing water depth along the vertical gradient (**Publication II**, Table 4.1). Additionally, the two abiotic parameters followed a seasonal pattern (**Publications II** and **III**, Tables 4.1 and 5.1). Generally, the following hypotheses were addressed: **(1)** macroalgal membrane fatty acid profiles do respond to variations in environmental conditions, prevailing along different gradients and

(2) adjustments in fatty acid composition are a major determinant in macroalgal photoacclimation and -protection.

7.1 Photosynthetic performance of macroalgae in variable environments

In studies dealing with macroalgal stress ecophysiology, measurements of variable chlorophyll *a* (Chl *a*) fluorescence of photosystem II (PSII) with a pulse amplitude-modulated (PAM) fluorometer have been widely applied in order to get a good first impression on the algal photosynthetic performance under the prevailing environmental conditions. Thereby, the maximum quantum yield (F_v/F_m) of PSII in dark-adapted plant or algal samples is the most often used chlorophyll fluorescence parameter (Büchel and Wilhelm 1993, Maxwell and Johnson 2000, Hanelt and Nultsch 2003). It can be described by the following equation: $F_v/F_m = (F_m - F_0) / F_m$, with the variable fluorescence (F_v) representing the difference between the maximal fluorescence (F_m), when all PSII reaction centers are reduced (“closed”), and the dark-adapted initial minimal fluorescence (F_0), when all PSII reaction centers are oxidized (“open”; Schreiber et al. 1994, Hanelt 1998). Since the D1 reaction center protein of PSII is supposed to be one of the most stress-susceptible component of the photosynthetic machinery (Mattoo et al. 1984, Ohad et al. 1984, Andersson et al. 1992, Barber and Andersson 1992, Demmig-Adams and Adams 1992, Aro et al. 1993, Park et al. 1996), photoinhibition or -damage caused by unfavorable abiotic conditions is directly reflected by a decrease of F_v/F_m values. Consequently, the maximum quantum yield can be considered as a sensitive marker of physiological fitness and, further, as indicator for stress experienced by the alga (Maxwell and Johnson 2000). For example, Edwards and Kim (2010) showed that the maximum quantum yield of surface blades of *Macrocystis pyrifera* in the Point Loma kelp forest located near San Diego, California, USA mirror the irradiance climate, to which the blades are exposed during the course of a day. In the early morning hours, F_v/F_m values were high, but they declined as the irradiance increased during the day and reached their minimum at midday, when the light-induced stress was highest. Following the decrease of the irradiance during the afternoon, F_v/F_m recovered again and returned to the initial high values at around sunset.

In general, we observed that the maximum quantum yields of the brown and red macroalgal species studied were within the typical high range reported for non-stressed algae (**Publications I-III**), which span from 0.7 to 0.8 in Phaeophyceae and from 0.6 to 0.7 in Rhodophyta (absolute values of F_v/F_m are only given for *M. pyrifera*, Publication II, Table 4.2; e.g., Büchel and Wilhelm 1993, Hanelt et al. 1993). The ability of the algae to maintain their maximum quantum yields within these ranges indicates that the photosynthetic apparatus showed no signs of inhibition and, thus, was not considerably negatively affected by any of the different abiotic environments (Maxwell and Johnson 2000). Additionally, we found

consequent adjustments in photosynthetic characteristics, namely photosynthetic capacity (ETR_{max}) and saturating irradiance (E_k), of the investigated kelp species. On the geographic scale, *Lessonia berteroana*, which shows a more northern distribution (16°S-30°S), exhibited higher values of ETR_{max} and E_k than *Lessonia spicata*, which occurs further south (29°S-40°S) (**Publication I**, Figure 3.4). Along the depth gradient, the photosynthetic capacity and saturating irradiance of *M. pyrifera* fronds tended to be higher in shallow than in deep waters. This response was found during long-term and short-term depth acclimation (**Publication II**, Table 4.2). A similar result was detected by Gerard (1986) and Colombo-Pallotta et al. (2006), who found a decline of both photosynthetic characteristics in *M. pyrifera* blades with increasing water depth. Furthermore, we observed that ETR_{max} and E_k of *M. pyrifera* followed a seasonal pattern, with higher values in summer than in winter (**Publication II**, Table 4.2). Generally, all of these adjustments in photosynthetic characteristics followed the well-known pattern of photoacclimation to different light climates, displaying higher photosynthetic capacities and saturating irradiances in high-irradiance environments compared to low-irradiance environments. This allows the algae to optimize their photosynthetic performance under variable environmental conditions. A greater photosynthetic capacity and saturating irradiance is beneficial in high-irradiance habitats, because it allows for protection of the photosynthetic apparatus against high light intensities. Lower values of ETR_{max} and E_k, in contrast, are favorable in low-irradiance environments, since they enable algal photosynthesis to be saturated already at lower light intensities (Reiskind et al. 1989, Marquardt et al. 2010).

Overall, the results of the three studies reveal a generally high photosynthetic plasticity of the investigated macroalgal species to the various abiotic conditions, prevailing along the spatial and temporal gradients, evidenced by constantly high maximum quantum yields in the range of non-stressed algae (Büchel and Wilhelm 1993, Hanelt et al. 1993). The maintenance of high F_v/F_m values is only possible, if severe photoinhibition and -damage is prevented through efficient photoacclimatory and -protective mechanisms, which are discussed in the following sections (Chapters 7.2 and 7.3).

7.2 Macroalgal photoacclimatory and -protective strategies along spatial gradients of environmental factors

Various photoacclimatory and -protective mechanisms enable the investigated macroalgal species to survive and grow under variable environmental conditions, which can be found along the different latitudinal and vertical gradients. In the next sections, first, the significance of photosynthetic pigments (including the xanthophyll cycle), phlorotannins and antioxidants is considered, followed by an evaluation of the role of adjustments in membrane lipid composition in photoacclimation.

7.2.1 Ecophysiological plasticity: photosynthetic pigments, phlorotannins and antioxidants

In general, the results of the present dissertation show that the studied macroalgae adjusted their ecophysiological status in terms of photosynthetic pigments (including the xanthophyll cycle), phlorotannins and antioxidants to the prevailing abiotic conditions along the latitudinal and vertical gradients.

We found that the algal pigment profiles followed the classical pattern of acclimation to various light conditions along the spatial gradients, generally exhibiting lower pigment concentrations under high-light regimes and greater pigment concentrations under low-light regimes (**Publications I**-**III**) (Falkowski and LaRoche 1991). Moreover, we observed adjustments with respect to the photoprotective xanthophyll cycle (**Publications I** and **II**). On the geographical scale, the de-epoxidation state (DPS) of the xanthophyll cycle pigments, which represents a measure for the rate of pigment conversion, of the two cryptic species of the *Lessonia nigrescens* complex tended to be higher at greater levels of solar radiation, prevailing at lower latitudes (**Publication I**, Figure 3.3b). Similarly, during long- and short-term depth exposure, contents of chlorophyll *c* (Chl *c*) and fucoxanthin in fronds of *Macrocystis pyrifera* rose, whereas the DPS of the xanthophyll cycle pigments declined with increasing water depth (**Publication II,** Table 4.3). These findings were in line with the observations of some previous studies, which compared the photosynthetic pigment composition in blades of *M. pyrifera* from different depths levels (Wheeler 1980, Gerard 1986, Smith and Melis 1987, Colombo-Pallotta et al. 2006, García-Mendoza and Colombo-Pallotta 2007). There, it was detected that blades from deeper water layers possess greater amounts of antenna pigments (Chl *c*, fucoxanthin) (Smith and Melis 1987, Colombo-Pallotta et al. 2006) and lower sizes of the xanthophyll cycle pigment pool (VAZ) as well as rates of xanthophyll cycle pigment conversion (Colombo-Pallotta et al. 2006, García-Mendoza and Colombo-Pallotta 2007) than blades from surface layers. Respective acclimations in pigment contents to the new prevailing light climates were also found in several brown algal species (*Ascophyllum nodosum*, *Fucus vesiculosus*, *M. pyrifera*, *Nereocystis luetkeana*) after the transplantation to various water depths (Duncan 1973, Ramus et al. 1977, Wheeler 1980, Gerard 1986). Additionally, large outdoor mesocosm studies conducted along the Chilean coast showed that floating individuals of *M. pyrifera* adjusted to the high light intensities at the sea surface by lowering their pigment contents (Rothäusler et al. 2011a, 2011b). Besides the acclimatory responses in kelp species, we also observed that the red alga *Chondrus crispus*, which inhabits a lower position on the coast of Helgoland, exhibited significantly greater Chl *a* concentrations than the red alga *Mastocarpus stellatus*, which lives at higher shore levels (**Publication III**, Table 5.2).

Overall, our results related to photosynthetic pigments show that the investigated macroalgal species favored photoprotection in high-irradiance environments and enhanced light-harvesting in low-irradiance environments along steep gradients (**Publications I**-**III**). Thereby, high chlorophyll and antenna pigment contents allow the algae to absorb more photons at low light levels, so that light utilization becomes more efficient. Vice versa, lower pigment concentrations protect the algal photosynthetic apparatus against excessive absorption of irradiance under high light conditions (e.g., Wheeler 1980, Smith and Melis 1987). Additionally, our findings confirm that the xanthophyll cycle is crucial as photoprotective mechanism under high irradiance conditions in the tested brown macroalgal species (**Publications I** and **II**). The xanthophyll cycle is activated by the light-driven acidification of the thylakoid lumen and involves the conversion of violaxanthin, which contains two epoxy-groups, to the epoxy-free zeaxanthin. During these two de-epoxidation steps the dissipation of excessively absorbed excitation energy by non-photochemical quenching (NPQ) is enhanced, so that photodamage of the photosynthetic reaction centers is prevented (Pfündel and Bilger 1994, Goss et al. 2005). This was for example shown by Colombo-Pallotta et al. (2006), who found a correlation between the VAZ and the NPQ capacity in blades of *M. pyrifera* from Ensenada, Baja California, Mexico. Blades collected near the water surface exhibited a high VAZ as well as a great NPQ capacity, whereas blades from deeper depths showed the opposite pattern. Similarly, the two kelp species *Laminaria digitata* (shallow-water alga) and *Laminaria abyssalis* (deep-water alga) adjusted their NPQ capacities via changes of the photoprotective pigment pool to the prevailing light climates at the different depth levels (Rodrigues et al. 2002). Under unfavorable environmental conditions, particularly high levels of irradiance, the de-epoxidation reactions of the xanthophyll cycle can be activated within several minutes (Pfündel and Bilger 1994). Firstly, it is suggested that the xanthophyll cycle occupies an important role in macroalgal photoacclimation along the gradient of solar radiation on the geographical scale (**Publication I**, Figure 3.3). Secondly, it was also shown that this photoprotective mechanism is particularly important in macroalgae inhabiting shallow water layers, since these habitats are characterized by abrupt changes of environmental conditions **(Publication II**, Table 4.3; Lüning and Dring 1979). Additionally, as already observed for the pelagic *Sargassum natans* (Schofield et al. 1998), we propose that the rapid activation of the xanthophyll cycle allows algae to tolerate the sudden exposure to very high light intensities, when they detach from their primary substratum and float to the sea surface (Rothäusler et al. 2011c; see Chapter 7.5 for details).

Our findings suggest further that the antioxidant activity of the investigated macroalgae is enhanced, when the species are exposed to unfavorable environmental conditions, particularly high irradiances of photosynthetically active (PAR) and ultraviolet (UV) radiation,

prevailing, for example, in surface water layers (**Publications II** and **III**). During long-term depth exposure, fronds of *M. pyrifera* developed higher antioxidant activities in shallow than in deeper waters. Short-term depth acclimation of the giant kelp during austral summer was in line with this observation. Here, fronds, which were transplanted from 4 m to 0.2 m water depth for 14 days, responded to the new abiotic environment with a considerable increase in antioxidant activity (**Publication II**, Figures 4.1c and 4.3b). Similar results were reported in a study by Cruces et al. (2012), in which the three South Pacific kelp species *Durvillaea antarctica*, *M. pyrifera* and *Lessonia spicata* exhibited highest radical scavenging activities after UV-exposure at elevated temperature (20°C). These environmental conditions are comparable to those found in surface waters during austral summer in our transplantation experiment. Furthermore, we detected that the antioxidant activity was generally higher in *M. stellatus* than in *C. crispus* (**Publication III**, Figure 5.2). Similarly, Collén and Davison (1999) found species-specific differences with respect to the efficiency of the reactive oxygen metabolism, with greater values in *M. stellatus* compared to *C. crispus*. Antioxidants work as a defense system against damages induced by reactive oxygen species (ROS), whose formation is stimulated during the exposure to environmental stress factors (Mallick and Mohn 2000). Adverse effects of ROS can be manifold. For example, peroxidation of membrane lipids might result in the formation of aldehydes, what finally might cause membrane leakage (Apel and Hirt 2004, Halliwell and Gutteridge 2015). Consequently, thylakoid membrane integrity might not be maintained and stress-induced repair processes of membranes might be slowed down, resulting in severe decreases of photosynthetic capacity (Bischof and Rautenberger 2012).Therefore, we suggest that the more effective defense system against ROS found in algal species living in shallower water depths may lead to an general increase in stress tolerance (Collén and Davison 1999), which allows the algae to withstand the potentially challenging environmental conditions in their habitats.

As for the antioxidants, we observed that the tested kelp species exhibited greater phlorotannin concentrations, when they live in habitats characterized by high levels of solar radiation (**Publications I** and **II**). A similar acclimatory pattern was detected by Cruces et al. (2012), who showed for various South Pacific kelp species that the concentration of total soluble phlorotannins is correlated with the antioxidant activity. This correlation exists probably due to the fact, that, amongst others, phlorotannins are thought to bear an antioxidative function (Koivikko et al. 2005). Along the latitudinal gradient, phlorotannins displayed greater contents in *Lessonia berteroana* compared to *Lessonia spicata* (**Publication I**, Figure 3.2). Along the vertical gradient, levels of phlorotannins in fronds of *M. pyrifera* decreased with increasing water depth. However, this acclimation response could only be observed during long-term depth exposure in austral winter (**Publication II**, Figure 4.3a). Generally, the formation of phlorotannins is a widely observed response of brown

macroalgae, like *Fucus* spp., *L. spicata* and *M. pyrifera*, to high levels of UV and/or PAR, suggesting the potential role of these compounds in high irradiance defense (Swanson and Druehl 2002, Schoenwaelder et al. 2003, Henry and van Alstyne 2004, Gómez and Huovinen 2010). Accordingly, we propose that the higher phlorotannin levels found in *L. berteroana* at lower latitudes and in *M. pyrifera* in surface waters protect those specimens from the harmful effects of high solar radiation prevailing in their respective habitats. Additionally, since phlorotannins are also thought to function as herbivore deterrence (Koivikko et al. 2005), a larger amount of them might allow the further north distributed species *L. berteroana* to tolerate the potentially higher grazing pressure, which was found to increase at lower latitudes (Broitman et al. 2001).

Overall, the results of the three publications of this dissertation confirm that adjustments in photosynthetic pigment profiles, xanthophyll cycle and antioxidant activities as well as phlorotannin concentrations form part of the suite of macroalgal photoacclimatory and -protective mechanisms to the various abiotic conditions, particularly variations in the irradiance climate, to which the algae are exposed along the different spatial gradients. In addition to this, new insights according to the role of adjustments in membrane lipid composition in macroalgal photoacclimation and -protection are provided in the following sections (Chapters 7.2.2 and 7.3).

7.2.2 Adjustments in total lipids and membrane fatty acid composition

The total lipid contents of the studied brown and red macroalgae were relatively low compared to those of microalgae, representing approximately 1.5-3.0% of dry weight (**Publications I**-**III**, Tables 3.2 and 5.2, Figures 4.1e and f and 4.3c). These results are in line with the lipid levels found for various macroalgal species (e.g., Jensen 1993, Fleurence et al. 1994, Sánchez-Machado et al. 2004a, Ortiz et al. 2009, Kumari et al. 2010) and Herbreteau et al. (1997) proposed that very low total lipid contents appear to be characteristic for plants living in marine environments. However, we observed that the polyunsaturated fatty acids (PUFA) made up more than 50 mass% of the total fatty acids (**Publications I**-**III**, Tables 3.2, S4.5 and 5.2). Furthermore, approximately 45-60 mass% of the brown algal PUFA fraction (**Publications I** and **II**, Tables 3.2 and S4.5) and even up to 75 mass% of the red algal PUFA fraction (**Publication III**, Table S5.1) was composed of n-3 PUFA, which are defined as polyunsaturated fatty acids with the first double bond being located at the third carbon atom, counted from the methyl end. Previous investigations detected similar high PUFA levels in a multitude of macroalgae (e.g., Fleurence et al. 1994, van Ginneken et al. 2011, Miyashita et al. 2013, Schmid et al. 2014), which might make them useful for industrial applications (see Chapter 7.5 for details).

The detailed characterization of the fatty acid composition of algae is thought to be an adequate approach to get a general idea about the degree of the fatty acid saturation or unsaturation of a given algal species. To the best of our knowledge, we described the fatty acid profiles of some of the investigated macroalgae, namely the two cryptic seaweed species *Lessonia berteroana* and *Lessonia spicata* as well as the rhodophyte *Mastocarpus stellatus*, for the first time in the present dissertation (**Publications I** and **III**, Tables 3.2 and S5.1). Generally, the fatty acid compositions found in these species were in good agreement with other results reported for various members of the Phaeophyceae and Rhodophyta, respectively, suggesting that the lipid profiles are linked to the taxonomic classification of macroalgae (e.g., Fleurence et al. 1994, Graeve et al. 2002, Khotimchenko et al. 2002, Hanson et al. 2010, Galloway et al. 2012). Additionally, an in-depth knowledge of algal fatty acid profiles is essential for further studies on lipid metabolism and on the effect of environmental factors on lipid composition.

The fluidity of a biomembrane is determined by its fatty acid composition, particularly in terms of degree of fatty acid saturation and chain length. Membranes containing higher amounts of saturated and/or longer-chain fatty acids are more rigid, whereas membranes with higher levels of unsaturated and/or shorter-chain fatty acids exhibit a greater fluidity. A proper cell functioning in macroalgae is only guaranteed, if the membrane fluidity is optimal (Buchanan et al. 2000). However, changes in environmental conditions are known to influence the fluidity of membranes. For example, high temperature causes fluidization, which eventually leads to a disintegration of the lipid bilayer and, thus, favors membrane leakage. Low temperature, in contrast, gives rise to an enhanced rigidity of membranes. Finally, all these alterations result in a non-optimal membrane fluidity and, hence, bear the potential to cause reduced photosynthetic and carbon assimilation rates, which ultimately leads to cell dysfunction as well as limited survival of the algae (Los and Murata 2004, Eggert 2012). Consequently, it is crucial for macroalgae to maintain their thylakoid membranes intact and operative over a wide range of abiotic factors, so that an efficient functioning of the photosynthetic machinery is guaranteed (Somerville and Browse 1991, Becker et al. 2010 and references therein). It is assumed, that this requires readjustments of the membrane fluidity through changes of the fatty acid composition in accordance with the environmental conditions of the algal habitats (Sanina et al. 2008). Some previous investigations have demonstrated that variations in levels of temperature (e.g., Pettitt et al. 1989, Al-Hasan et al. 1991, Dawes et al. 1993, Sanina et al. 2008) or light (e.g., Pettitt and Harwood 1989, Floreto and Teshima 1998, Hotimchenko 2002, Khotimchenko and Yakovleva 2005) have an impact on macroalgal fatty acid profiles. Thereby, adjustments of the degree of saturation were mainly observed (e.g., Sato and Murata 1981, Pettitt and Harwood 1989, Khotimchenko and

Yakovleva 2005, Guihéneuf et al. 2009, Becker et al. 2010), but alterations with respect to fatty acid chain length were also found (e.g., Sato et al. 1979 and references therein). However, due to the mainly non-consistent results of these studies, it was barely impossible to generalize lipid-related responses of macroalgae to certain changes in the abiotic environment.

Generally, since marine macroalgae are poikilothermic organisms, the effect of variation in temperature on their membrane fatty acid profiles is plausible, but changes in the membrane fluidity in response to alterations in light conditions are less understandable (Klyachko-Gurvich et al. 1999). However, in photosynthetic organisms exists a close connection between lipids and the photosynthetic integral membrane protein complexes, which are anchored within the thylakoid membranes (Raven et al. 2005). Moreover, recent studies on molecular structures of cyanobacterial photosystems detected that lipids are not only important for the formation of the lipid bilayer, but also for the establishment and maintenance of the structures of the photosynthetic protein complexes (Kruse et al. 2000, Jones 2007, Loll et al. 2007, Kern et al. 2009). It was shown, for example, that lipids form a belt around the D1 reaction center protein that provides a flexible environment for the exchange of the protein during the D1 protein repair cycle. Additionally, lipids were found to fill intra-protein cavities, through which quinones can diffuse, and to bind light harvesting cofactors (Jones 2007, Loll et al. 2007). Although this information stems from data on cyanobacterial reaction centers, it is reasonable to believe that lipids in algae serve comparable functions, because the lipid composition of thylakoids is generally highly conserved among all oxygenic photosynthetic organisms (Loll et al. 2007, Goss and Wilhelm 2009). Consequently, light-induced variation in the photosynthetic performance might likely be mirrored in the thylakoid membrane fatty acid composition (e.g., Pettitt and Harwood 1989). Thereby, adjustments of fatty acid profiles can facilitate electron and ion transport across/within the thylakoid membranes (Klyachko-Gurvich et al. 1999) and enhance the stabilizing effect of lipids on the protein complexes during photosynthesis under variable environmental conditions (Hölzl et al. 2005, Mizusawa and Wada 2012).

Overall, the total lipid contents and fatty acid profiles of the tested macroalgae responded to variations in environmental conditions, prevailing along the spatial gradients. However, adjustments of these biochemical parameters were only observed along the depth gradients (**Publications II** and **III**), but not on the geographic scale (**Publication I**). Additionally, the responses were further influenced by season (see Chapter 7.3 for further information).

Despite the clear differences in solar radiation and sea surface temperature between the different sampling sites of algal individuals along the northern-central Chilean coast (see

Chapter 3 for details), the total lipid contents and saturation states of fatty acids of *L. berteroana* and *L. spicata* did not differ with latitude or species (**Publication I**, Table 3.2). Consequently, we assume that adjustments in total lipids and fatty acid composition play a negligible role in the photoacclimatory and -protective strategies of these two cryptic kelp species. Additionally, they seem to be insignificant for the species separation within the *Lessonia nigrescens* complex along the northern-central Chilean coast. Although Khotimchenko et al. (2002) proposed that habitat conditions rather than the geographical location determine differences in macroalgal fatty acid composition, our results are contradictory to those observed by Sánchez-Machado et al. (2004a, 2004b) and Colombo et al. (2006). These authors found variations in total lipids and fatty acid profiles of macroalgae with respect to latitude. Sánchez-Machado et al. (2004a, 2004b) reported that macroalgae inhabiting cold environments have significantly greater lipid levels than tropical specimens. It was also detected that cold-water species exhibit a higher degree of total unsaturation and are particularly richer in PUFA contents compared to tropical species. These differences in lipid composition were mainly attributed to the latitudinal variation in water temperature (Colombo et al. 2006).

In contrast to the results related to the geographical scale, we observed that rearrangements of lipid profiles of the investigated brown and red macroalgal species seem to be in fact important in photoacclimation and -protection along vertical gradients of environmental factors. Generally, we found higher total lipid contents in habitats with lower levels of irradiance (and temperature) than in habitats with higher levels of irradiance (and temperature) (**Publications II** and **III**). During long-term depth exposure in austral summer, fronds of *Macrocystis pyrifera* developed greater amounts of lipids in deep than in shallow waters (**Publication II**, Figure 4.3c). Similar depth-related total lipid patterns were observed in the two rhodophytes, with higher contents in the lower-shore species *Chondrus crispus* compared to the higher-shore species *Mastocarpus stellatus* (**Publication III**, Table 5.2). Although some micro- and macroalgal (*Costaria costata*, *Cyclotella meneghiniana, Grateloupia turuturu*, *Tichocarpus crinitus* and *Ulva lactuca,* formerly *U. fenestrata*) species were shown to exhibit enhanced total lipid contents at low light conditions (Sicko-Good et al. 1988, Hotimchenko 2002, Khotimchenko and Yakovleva 2005), our results are contradictory to those found by Ito and Tsuchiya (1977), who detected a decline of total lipid contents with increasing water depth in various brown and green macroalgae.

In addition to the depth-related variation in total lipid contents, we also observed differences in the degree of fatty acid saturation and chain length along the vertical gradients. Thereby, the kelp *M. pyrifera* and the rhodophytes *M. stellatus* and *C. crispus* showed contrasting acclimation patterns (**Publications II** and **III**). Fronds of *M. pyrifera* possessed a greater degree of saturation in surface water layers than in greater depths (**Publication II**,

Figures 4.1h and 4.3d). This acclimation response was present during long-term depth exposure in austral summer (**Publication II**, Figure 4.3d) and during the short-term transplantation experiment in austral winter (**Publication II**, Figure 4.1h). Additionally, a switch from shorter- to longer-chain fatty acids was found in *M. pyrifera* fronds after they were transplanted from 4 m to 0.2 m water depth in winter (**Publication II**, Figure 4.4). We assume that the observed adjustments in the degree of fatty acid saturation might act as compensation for temperature-induced changes of the membrane fluidity in *M. pyrifera*, prevailing at the different water depths. In doing so, the giant kelp is able to maintain an optimal membrane fluidity in different abiotic environments along the vertical gradient (Somerville and Browse 1991, Becker et al. 2010 and references therein). This explanation might be particularly true in austral summer, when temperature differences between the various depth levels were more pronounced than during winter (for details see Table 4.1). Thereby, the enhanced saturation in shallow water layers is thought to counteract the higher temperature-caused fluidization of the membranes. On the other hand, the greater unsaturation in deeper depths is proposed to compensate for the increased rigidity of membranes, which is induced by the lower temperature in these water layers (Harwood 1994, Murata and Los 1997, Buchanan et al. 2000). The same adjustments in fatty acid composition as a response to a change in temperature have been reported for cyanobacteria, micro- and macroalgae (e.g., Sato and Murata 1981, Pettitt et al. 1989, Wada and Murata 1990, Al-Hasan et al. 1991, Thompson et al. 1992, Dawes et al. 1993, Renaud et al. 2002, Sushchick et al. 2003, Becker et al. 2010), and are thought to be one of the generally accepted mechanisms of temperature acclimation in marine organisms (Al-Hasan et al. 1991, Dawes et al. 1993, Klyachko-Gurvich et al. 1999).

Furthermore, we suggest that differences in the membrane fatty acid composition along the depth gradient might mirror the light-induced adjustments of the thylakoid composition in *M. pyrifera*. In line with our observations, an elevated degree of unsaturation was found in various algal species (e.g., *C. meneghiniana*, *Pavlova lutheri*, renamed as *Diacronema lutheri* and *T. crinitus*), when these were exposed to low light conditions (Sicko-Good et al. 1988, Khotimchenko and Yakovleva 2005, Guihéneuf et al. 2009, Wacker et al. 2015). Since thylakoid membranes contain high amounts of unsaturated fatty acids, particularly PUFA (Al-Hasan et al. 1991, Floreto and Teshima 1998, Miyashita et al. 2013), this rise in unsaturation was attributed to a low light-induced increase of the thylakoid surface (e.g., stacking of thylakoids in phytoplankton). In doing so, the capture of photons in low-irradiance regimes is enhanced (Sukenik et al. 1989, Wacker et al. 2015). Similarly, we propose that the higher degree of unsaturation helps *M. pyrifera* to enhance the efficiency of its light-harvesting properties through an increase in the production of thylakoid membranes and, thus, optimize its photosynthetic performance under low light conditions in deeper waters. High antenna

pigment contents, which were found in fronds of *M. pyrifera* in this low-light habitat (**Publication II**, Table 4.3), are in line with this assumption.

We further suggest that the degree of fatty acid saturation might also influence the activity of the xanthophyll cycle in *M. pyrifera*. Vieler et al. (2008) found a dependence of the xanthophyll cycle in spinach on the phase transition of membrane lipids. Lipids exist in two different physical states, as a gel or as a fluid. Increases in temperature lead to the conversion from the gel to the fluid phase, a process known as phase transition. The precise temperature, at which the conversion occurs, is called melting temperature. It is determined by the lipid structure and, thus, differs from lipid to lipid (Buchanan et al. 2000). Furthermore, the melting temperature can be altered through adjustments of the membrane fatty acid composition. Greater levels of saturation enhance the melting temperature and favor the gel lipid phase, whereas higher levels of unsaturation lower the temperature and support the fluid lipid phase (Chapman 1975, Buchanan et al. 2000). Generally, it was shown in the thylakoids of spinach that the phase transition from the gel to the liquid-crystalline phase influences both the solubilization of the substrate violaxanthin and the activity of the enzyme violaxanthin de-epoxidase (VDE), which catalyzes the de-epoxidation reaction of violaxanthin to zeaxanthin through the intermediate antheraxanthin. At higher temperatures, the gel phase provides the ideal environment for the activity of VDE, whereas at lower temperatures, the liquid-crystalline phase is better suited to maintain a high level of de-epoxidation activity (Vieler et al. 2008). Based on these results, we assume that the enhanced degree of fatty acid saturation in *M. pyrifera* fronds in shallow waters (**Publication II**, Figures 4.1h and 4.3d) might be favorable for an highly active xanthophyll cycle. By increasing the melting temperature, the saturation promotes the gel lipid phase of thylakoid membranes and, hence, finally provides an optimal environment for the activity of the xanthophyll cycle in a habitat, that is, particularly in summer, characterized by higher water temperatures (for details see Table 4.1). This suggestion is supported by our observations related to the depth-dependence of the xanthophyll cycle in *M. pyrifera*. There, we detected that the xanthophyll cycle pigment de-epoxidation of *M. pyrifera* was highest in surface waters and decreased with increasing water depth (**Publication II**, Table 4.3).

In contrast to the results observed for the giant kelp, the two tested rhodophytes showed the opposite acclimation pattern related to lipid profiles along the vertical gradient on the coast of Helgoland. *Mastocarpus stellatus*, which lives at higher shore levels, was richer in unsaturated and shorter-chain fatty acids, whereas saturated and longer-chain fatty acids were predominant in *C. crispus*, which inhabits a lower position on the coast (**Publication III**, Table 5.2). This might indicate that there is no need for *M. stellatus* to favor fatty acid saturation in order to prevent an excessive fluidization of its membranes due to the greater levels of temperature (and irradiance), prevailing at higher shore habitats. Instead, this

species incorporates great amounts of fatty acids with lower melting points into its membranes, so that the membrane fluidity is even enhanced (Buchanan et al. 2000). Previous studies detected similar acclimatory responses in the macroalgae *Analipus japonicus* (formerly *Heterochordaria abietina*), *Saccharina religiosa* (formerly *Laminaria religiosa)*, *Palmaria palmata*, *Ulva australis* (formerly *U. pertusa*) and *Undaria pinnatifida* with respect to habitat depth, with a higher degree of unsaturation in shallower compared to deeper waters (Ito and Tsuchiya 1977, Becker et al. 2010). Furthermore, in some red algae, fatty acid unsaturation was found to be stimulated by an increase in light intensity (Pettitt and Harwood 1989). We suggest that the greater degree of unsaturation in *M. stellatus* might also be attributable to the elevated light levels in its high-shore habitat on the island of Helgoland. These shallower water layers are characterized by extremely variable environmental conditions, which have the potential to be stressful for organisms living there (Bell 1993, Kübler and Davison 1993, Dring et al. 1996, Sagert et al. 1997, Collén and Davison 1999). Generally, a high amount of PUFA, which represent the bulk of unsaturated fatty acids in *M. stellatus*, is thought to be favorable in unsteady habitats. Polyunsaturated fatty acids are most responsive to environmental changes, so that they can adequately react to changes in the abiotic environment (Nelson et al. 2002). Under these conditions, the formation of ROS is promoted, which in turn might favor the degradation of the D1 reaction center protein of PSII. More fluid membranes facilitate the D1 protein repair cycle (Becker et al. 2010 and references therein) and support the ion and electron transport between the two photosystems (Klyachko-Gurvich et al. 1999, Jones 2007, Guihéneuf et al. 2009). Consequently, we assume that higher levels of fatty acid unsaturation might help *M. stellatus* to maintain biomembranes operative in its highly variable habitat.

Not only along spatial gradients, but also during the course of a year, macroalgae are exposed to great fluctuations in solar radiation and temperature (Hurd et al. 2014). Consequently, there is evidence for temporal variability of algal fatty acid profiles on a seasonal scale (see Chapter 7.3 for further information).

7.3 Temporal variability of photoacclimation and -protection of macroalgae related to total lipids and membrane fatty acid composition

As for the vertical gradients of environmental factors, we observed differences in total lipids as well as in the degree of fatty acid saturation and chain length in the investigated algal species between various seasons (**Publications II** and **III**). The giant kelp *Macrocystis pyrifera* exhibited higher total lipid contents in austral summer than in austral winter (**Publication II**, Figures 4.1e and f and 4.3c), whereas season had no effect on the lipid levels in the two rhodophytes *Mastocarpus stellatus* and *Chondrus crispus* (**Publication III**, Table 5.2). The results of previous investigations on seasonal differences in total lipids were

very diverse. Schmid et al. (2014) found greater concentrations of total lipids in some macroalgae in summer and autumn (warmer seasons) than in winter and spring (colder seasons), whereas other authors observed the opposite pattern (e.g., Nelson et al. 2002, Blažina et al. 2009, Gerasimenko et al. 2011, Gosch et al. 2015). And yet others reported no variation in macroalgal total lipids with respect to season (Renaud and van Luong 2006, Denis et al. 2010, Gerasimenko et al. 2010, Schmid et al. 2014). We suggest that the higher lipid contents in *M. pyrifera* during summer might be attributable to a high light-induced increase in fatty acid synthesis. The reasons for a general rise in fatty acid synthesis under high-light regimes are not entirely clear. However, photosynthesis might cause these changes by enhancing the need for thylakoid membrane replacement (Pettitt and Harwood 1989). This membrane replacement, in turn, might be provoked by ROS-induced peroxidation of thylakoid membrane lipids and subsequent formation of aldehydes, which was shown to be enhanced under excessive irradiance conditions (Bischof et al. 2002, 2003, 2006, Dummermuth et al. 2003). Furthermore, algae were found to use light energy for the accumulation of triacylglycerols (TAG, neutral storage lipids), which might result in generally higher total lipid concentrations. For example, these responses have been reported for some macroalgal species, such as *Fucus serratus*, *Saccharina japonica* (formerly *Laminaria japonica*), *Tichocarpus crinitus* and *Ulva lactuca* (formerly *U. fenestrata*) (Kim et al. 1996, Goncharova et al. 2004, Khotimchenko and Yakovleva 2005). Previous studies proposed that TAG are usually produced when the capacity of algae to utilize excess light energy is exceeded (Hu et al. 2008, Guihéneuf et al. 2009, Sharma et al. 2012). Since the formation of TAG requires considerable amounts of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH), this process might help the algae to diminish photodamaging effects of excessive irradiance levels. Under less stressful environmental conditions, the in TAG stored energy can be utilized again in various metabolic processes (Roessler 1990). Triacylglycerols are rich in saturated fatty acids (Roessler 1990, Goss and Wilhelm 2009), so that a higher degree of saturation, which was found during austral summer in fronds of *M. pyrifera* (see below), correlates well with our suggestion of TAG accumulation. However, future studies, in which the effect of season on various lipid classes of the giant kelp is investigated, are needed for confirmation.

Besides the observed seasonal variability in total lipids, we found a greater degree of fatty acid saturation in months with a warmer water temperature and an enhanced degree of unsaturation in months with a colder water temperature in all three tested algae (*M. pyrifera*, *M. stellatus* and *C. crispus*) (**Publications II** and **III**, Table 5.2, Figures 4.1g and h and 4.3d). The same patterns were found in a multitude of macroalgal species, like *Ahnfeltia tobuchiensis*, *Caulerpa racemosa*, *F. serratus*, *Grateloupia turuturu*, *Saccharina japonica* (formerly *Laminaria japonica*), *Sargassum pallidum*, *Spatoglossum macrodontum*, *Ulva*

lactuca (formerly *U. fenestrata*) and *Ulva lobata* (Kim et al. 1996, Nelson et al. 2002, Sanina et al. 2008, Blažina et al. 2009, Denis et al. 2010, Gosch et al. 2015) and are thought to be mainly related to the variability in temperature over the course of a year (Gerasimenko et al. 2011). In addition, since the observed responses of the fatty acid profile of *M. pyrifera* to variations in environmental conditions along the depth gradient were not consistent in austral summer and winter (**Publication II**, Figures 4.1g and h, 4.3d and 4.4), we propose that these responses were further impacted by season. We also detected that the two red algal species *M. stellatus* and *C. crispus* accumulated shorter-chain fatty acids in their lipids in warmer months (**Publication III**, Table 5.2). As already described for the vertical gradient (for details see Chapter 7.2.2), we suggest that the observed adjustments in the degree of fatty acid saturation might act as compensation for temperature-induced changes of the membrane fluidity in the investigated macroalgae, prevailing during the various seasons. In summer, a higher degree of saturation is thought to prevent the algal membranes from becoming too fluid (Buchanan et al. 2000). In winter, in contrast, an overly rigidity of the membranes is avoided by increased levels of unsaturation (Buchanan et al. 2000), so that, for instance, the D1 protein repair cycle is able to work efficiently at low temperatures (Becker et al. 2010 and references therein). Additionally, we propose that unsaturation of membrane lipids can stabilize the photosynthetic apparatus of the tested algae against photoinhibition in winter, which may occur during periods of high irradiance at low temperatures (Wise 1995, Lohrmann et al. 2004). Thereby, the recovery of PSII from photoinhibition was shown to be accelerated under a high degree of unsaturation. This effect of unsaturation on low-temperature tolerance was found in cyanobacteria and higher plants and is suspected to be applicable to algae (Gombos et al. 1994, Moon et al. 1995). Not only water temperature, but also the irradiance climate differs during the course of a year (Hurd et al. 2014). Thus, we assume that the seasonal variation in light might also affect the fatty acid composition of the investigated macroalgal species. First of all, we believe that the lower amount of unsaturated fatty acids in algal samples from summer and autumn can be attributed to the high-light sensitivity of PUFA. Under excessive irradiance levels, lipids, particularly those being rich in PUFA, are degraded through peroxidation, which finally results in decreased levels of total unsaturation (Cosgrove et al. 1987, Apel and Hirt 2004, Halliwell and Gutteridge 2015). Furthermore, similar to the observations along the depth gradient (for details see Chapter 7.2.2), elevated levels of unsaturation during winter might mirror the increased need for thylakoid membrane surface for the optimization of the light-harvesting efficiency, which can be related to the lower light intensities prevailing during this season (Sukenik et al. 1989, Wacker et al. 2015). In the giant kelp, *M. pyrifera*, seasonal variation in fatty acid saturation might further facilitate the xanthophyll cycle activity (for details see Chapter 7.2.2) (Vieler et al. 2008). At higher water temperatures in summer, saturation promotes the gel lipid phase

of thylakoid membranes. At lower water temperatures in winter, contrarily, unsaturation favors the fluid lipid phase of membranes (Chapman 1975, Buchanan et al. 2000). In doing so, an optimal environment for the functioning of the xanthophyll cycle is created under variable environmental conditions and, hence, a high xanthophyll cycle activity can be maintained around the year (Vieler et al. 2008).

7.4 Conclusions

The results of the present dissertation highlight the enormous acclimation potential of the investigated brown and red macroalgae *Lessonia berteroana*, *Lessonia spicata*, *Macrocystis pyrifera*, *Mastocarpus stellatus* and *Chondrus crispus* to varying environmental conditions, prevailing along spatial (latitudinal and vertical) and temporal (seasonal) gradients.

In the three publications, we confirmed that adjustments in photosynthetic pigments (including the xanthophyll cycle), phlorotannins and antioxidants feature a high significance in macroalgal photoacclimation and -protection (**Publications I**-**III**). We further observed that macroalgal total lipid contents and fatty acid composition, particularly related to the degree of fatty acid saturation and chain length, clearly responds to variations in light and temperature (see hypothesis 1). This might indicate that adjustments in these biochemical parameters also form part of macroalgal photoacclimatory and -protective mechanisms. Generally, those adjustments in fatty acid profiles were highly variable and species-specific. We found, for example, that the giant kelp *M. pyrifera* and the two rhodophytes *M. stellatus* and *C. crispus* applied opposite strategies in fatty acid acclimation along the depth gradients (**Publications II** and **III**), which are likely implicate similar advantages for the algal physiological performance.

Our results reveal seasonal variability in fatty acids (**Publications II** and **III**) and might further suggest that modulations of algal lipid profiles are negligible along latitudinal gradients (**Publication I**), whereas they seem to play an important role in depth acclimation (**Publications II** and **III**). However, further studies, which include a greater number of species belonging to the Chlorophyta, Phaeophyceae and Rhodophyta, are needed to prove whether the lack of lipid adjustments on the geographic scale is universally valid or is rather a species-specific characteristic within the *Lessonia nigrescens* complex. Furthermore, our findings might indicate that light has a stronger impact on fatty acid composition than expected (**Publications II** and **III**) and that adjustments of the fatty acid composition not only help the tested algae to optimize their membrane fluidities, but also to create ideal environments for the functioning of the xanthophyll cycle under variable environmental conditions, which is crucial for the avoidance of severe photodamage. Changes in fatty acid profiles also seem to play a key role in macroalgal short-term acclimation responses, which was shown during the 14-day transplantation experiment, conducted on *M. pyrifera*

(**Publication II**). On the whole, we propose consequently that adjustments in the fatty acid composition are a major determinant in macroalgal photoacclimation and -protection, exhibiting a similar importance as the other tested response variables (see hypothesis 2).

Overall, the present dissertation provided a broadened picture of macroalgal photoacclimation and -protection along various spatial and temporal gradients of environmental factors, particularly light and temperature. Our understanding for the significance of photosynthetic pigments, the xanthophyll cycle, phlorotannins and antioxidants was deepened and new insights with respect to modulations of lipid profiles were added. The newly gained knowledge might help to improve predictions on responses of macroalgae towards abiotic stresses and might be useful for economic applications (see Chapter 7.5 for details).

7.5 Ecological and economic implications

Changes in abiotic conditions caused by large-scale environmental variations, like El Niño Southern Oscillation (ENSO) events (Glynn 1988, Chavez et al. 1999, Cobb et al. 2003) or the ongoing climate change (e.g., global warming and ozone depletion; Solomon et al. 1986, Wiltshire et al. 2009, Manney et al. 2011) may pose new challenges to macroalgae. Since macroalgae represent key organisms of coastal ecosystems (Steneck et al. 2002), those variations bear further the potential to alter the dynamics of entire ecosystem. This may include changes in competition relationships and alterations of the primary productivity (Tegner and Dayton 1991, Graham et al. 2007) or chemical composition of macroalgae, which in turn might affect the nutritional value for grazers and the energy flow through higher trophic levels (Hessen et al. 1997, Hurd et al. 2014). Furthermore, ENSO events, whose frequency and severity is suggested to increase (Diaz et al. 2001), are reported to cause extensive kelp mortality due to increases in water temperature (Dayton and Tegner 1984, Ladah et al. 1999, Martínez et al. 2003, Edwards 2004). Consequently, predictions of algal responses to abiotic stresses are nowadays particularly crucial, since macroalgae are more and more exposed to challenging environmental conditions.

Additionally, the results of this thesis might also help to predict how positively buoyant macroalgae are affected by changes in their abiotic environment after detachment. For instance, sporophytes of *M. pyrifera* may become detached from the substratum by herbivore grazing or storms (Tegner and Dayton 1991, Dayton et al. 1992, Tegner et al. 1995), so that they are commonly found free floating in coastal waters (Macaya et al. 2005, Rothäusler et al. 2009, 2012). Transported by ocean currents and winds, these algae may stay afloat for more than 100 days (Hobday 2000a) and travel considerable distances (approx. 1000 km, with approx. 7 km day^{-1}; Harrold and Lisin 1989, Tegner et al. 1995, Hobday 2000b, Hernández-Carmona et al. 2006). Thereby, the floating algae continue to provide habitats and contribute to the connectivity between distant populations of associated organisms and

kelps themselves via the release of gametes or spores at the new sites (Thiel and Haye 2006, Fraser et al. 2009, Hinojosa et al. 2010). Besides their importance as dispersal vector, kelp rafts that sink, provide important organic inputs to the sea floor, which may significantly enhance the productivity of benthic communities (Vetter 1995, Harrold et al. 1998). Detachment and subsequent floating to the sea surface is followed by abrupt alterations in irradiance and temperature conditions (Rothäusler et al. 2011c). These sudden changes in the abiotic environment were mimicked by us during the short-term transplantation experiment, when fronds of *M. pyrifera* were transferred from deeper to surface water layers (**Publication II**). Generally, predictions, drawn from the finding of this experiment might be of high significance due to the fundamental ecological relevance of floating macroalgae.

Our findings related to the characterization of macroalgal lipid profiles might further provide a valuable basis for commercial applications. From an economic point of view, the use of macroalgae becomes very attractive in a multitude of industrial purposes (see Chapter 1 for details). One example is the role of algae in human nutrition (Buchholz et al. 2012). A sufficient supply with polyunsaturated fatty acids, particularly PUFA of the n-3 series, is suggested to play an important role in the prevention of certain health issues in humans, mainly cardiovascular and inflammatory diseases (Horrocks and Yeo 1999, Simopoulos 2002, Calder 2006, Bocanegra et al. 2009). Since essential n-3 PUFA cannot be synthesized by humans, they have to be ingested through the diet (Gerster 1998, Kumari et al. 2010, Pereira et al. 2012). Some studies proposed that an enrichment of the diet with PUFA of the n-3 series might be beneficial for human health (Horrocks and Yeo 1999, Simopoulos 2002, Calder 2006, Bocanegra et al. 2009), with recommendations ranging from 135 to over 1000 mg n-3 PUFA person^{-1} day^{-1} (Meyer et al. 2003). Consequently, the demand for n-3 PUFA is currently rising, also for vegetarian n-3 PUFA (Fajardo et al. 2007). Until now, the major commercial available source of n-3 PUFA is fish oil (e.g., Fajardo et al. 2007), but taking the accumulation of pollutants in fish and the ongoing depletion of edible fish populations into account (Worm et al. 2009, Myers and Worm 2003), the need for alternative natural sources of PUFA of the n-3 series had become more and more a subject of interest. Due to some biochemical (high n-3 PUFA concentrations; e.g., Fleurence et al. 1994, van Ginneken et al. 2011, Miyashita et al. 2013, Schmid et al. 2014) and ecological (e.g., large stocks in coastal waters and easy cultivation on a large scale) characteristics of macroalgae, their use as potential new source of n-3 PUFA is suggested (e.g., Colombo et al. 2006, van Ginneken et al. 2011, Pereira et al. 2012, Schmid et al. 2014). In general, our results confirm the suitability of the tested macroalgae as potential alternative source of n-3 PUFA. Thereby, we would propose to rather use red algae for this purpose, since they seemed to exhibit higher relative proportions of PUFA of the n-3 series (**Publication III**) compared to those of

brown algae (**Publications I** and **II**). Moreover, our results about changes of macroalgal fatty acid composition with respect to variations in environmental conditions might help to actively manipulate the lipid profiles of various macroalgal species in land-based aquaculture operations through changes of the culture conditions. In doing so, macroalgae with economically preferable lipid profiles (e.g., high amounts of PUFA) can be produced (Guihéneuf et al. 2009, Mata et al. 2016). A better knowledge about the seasonal variability of macroalgal fatty acid compositions might further allow for a more selective harvesting of algae. For example, based on our findings, month with a colder water temperature seem to be the most appropriate harvesting times for macroalgae rich in unsaturated fatty acids (**Publications II** and **III**).

7.6 Perspectives for future research

According to the implications of the three publications, the need for future experiments and improvement of laboratory analyses became apparent. First of all, future studies, which deal with the determination of macroalgal fatty acid profiles for ecophysiological questions or commercial applications, should improve lipid extraction techniques. Generally, the lipid extraction yield can be enhanced by the usage of longer extraction times, multiple repeated extraction cycles or higher solvent to biomass ratios (Fajardo et al. 2007, Suganya and Renganathan 2012). However, these adjustments are not always applicable, since they make the analytical procedure much more time-consuming and cost-intensive. In the present three studies, a modified lipid extraction technique established by Bligh and Dyer (1959) was chosen. This method is most commonly applied for a wide range of biological samples, since it includes the usage of an organic solvent mixture, containing a polar (methanol) and a non-polar (dichloromethane or chloroform) component. Such solvent mixtures are able to extract both polar and neutral lipids and, hence, are thought to result in generally higher extraction yields (Ryckebosch et al. 2012, Ambrozova et al. 2014, Schmid et al. 2016). However, it should also be considered that some studies (Lewis et al. 2000, Ryckebosch et al. 2012) detected an underestimation of the total lipid content in microalgae, when the method by Bligh and Dyer (1959) was used. Due to the generally lower total lipid contents in macroalgae compared to microalgae (e.g., Jensen 1993, Fleurence et al. 1994, Sánchez-Machado et al. 2004a, Ortiz et al. 2009, Kumari et al. 2010), an underestimation of lipid amounts in the first would be even more significant. Furthermore, this method is unsuitable, when extracted lipids should be used for food applications, because the solvents do not meet food grade standards (Schmid et al. 2016). Consequently, the need for alternative lipid extraction techniques becomes more and more a subject of interest. Since the extraction yield strongly depends on the species-specific distribution of polar and neutral lipids (D'Oca et al. 2011), it is difficult to find a method, which can be applied likewise to all macroalgal

species. This consideration further highlights the importance for a good understanding of the fatty acid profiles in a multitude of macroalgae.

In line with previous studies (e.g., Al-Hasan et al. 1991, Somerville and Browse 1991, Dawes et al. 1993, Floreto et al. 1993, Floreto and Teshima 1998, Khotimchenko and Yakovleva 2005), we observed highly inconsistent responses in macroalgal fatty acid profiles to variations in abiotic parameters, which underlines that fatty acid acclimation needs to be checked even more preciously in future investigations. First, future research should work with the lipid profiles of isolated thylakoids. In doing so, the results would only concentrate on abiotic change-induced variations in the lipid composition of photosynthetic membranes and would not be influenced by those of other cell organelles. This was shown for tobacco plants by Moon et al. (1995), who detected that the lipid analysis of isolated thylakoids provides more informative evidence than the analysis of lipids, which were directly extracted from leaves. Second, future studies should focus on the separation of total lipids into different lipid classes and the determination of fatty acid composition within each single lipid class. This would allow for the identification of which membranes (e.g., thylakoid membranes) are primarily affected by adjustments in fatty acids. It would also avoid that minor variations in fatty acids, which are only present in certain lipid classes, are overlooked (Guihéneuf et al. 2009). This might be of high interest, since previous investigations showed that different fatty acids were not evenly distributed amongst various lipid classes and that changes in the degree of fatty acid saturation depend on the composition of particular lipid classes (Kim et al. 1996, Goss and Wilhelm 2009, Guihéneuf et al. 2009). The two above described approaches are thought to lead to clearer results in terms of macroalgal responses in fatty acid profiles and their function in photoacclimation. Moreover, research on fatty acid elongases and desaturates as well as gene expression studies are suggested, in order to gain in-depth insights into the underlying processes of changes in macroalgal fatty acid composition (Ohlrogge and Browse 1995, Los 1997, Renaud et al. 2002, Guschina and Harwood 2006).

Apart from methodological considerations, further experiments are recommended. Field experiments, as applied in **Publication II** of this thesis, mimic the complex abiotic environment of macroalgae, with all its synergistic or antagonistic interactions of abiotic factors, best (Hurd et al. 2014). However, particularly with the chosen experimental set-up, field experiments make it very difficult to disentangle which environmental factor is responsible for the observed ecophysiological changes in macroalgae. Future fully controlled field or laboratory experiments, in which the effect of one abiotic parameter on macroalgal ecophysiology is investigated at a time, are needed to evaluate the relative influence of the different parameters. Generally, these experiments can further help to understand whether multiple abiotic factors might have impacted the observed macroalgal acclimation responses

simultaneously. In the present three studies, we only concentrated on variations in light and temperature, but other parameters could also differ at the various latitudes, water depths and seasons and thereby affect algal ecophysiology and previous studies reported, for instance, that changes in heavy metals (Kumar et al. 2010a), nutrients, like nitrogen (Opute 1974, Piorreck et al. 1984, Mishra et al. 1993) or phosphate (Reitan et al. 1994, El-Sheek and Rady 1995, Goss and Wilhelm 2009), pH (Guckert and Cooksey 1990), salinity (Takagi et al. 2006, Kumar et al. 2010b) and UV radiation (Hessen et al. 1997 and references therein) have the potential to influence the fatty acid profiles of cyanobacteria, micro- and macroalgae.

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Johann Wolfgang von Goethe (1749-1832)

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Ich, **Kristina Koch** (Bremer Str. 46, 28844 Weyhe; Matr.-Nr.: 2142479),

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