Facing Climate Change:

Physiological and biochemical responses of European kelp species to ocean warming

Nora Diehl

Dissertation

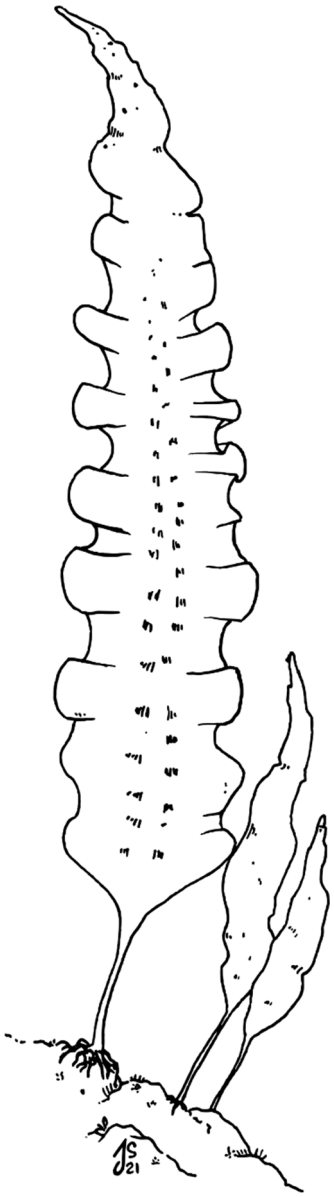
in fulfilment of the requirements for the degree of

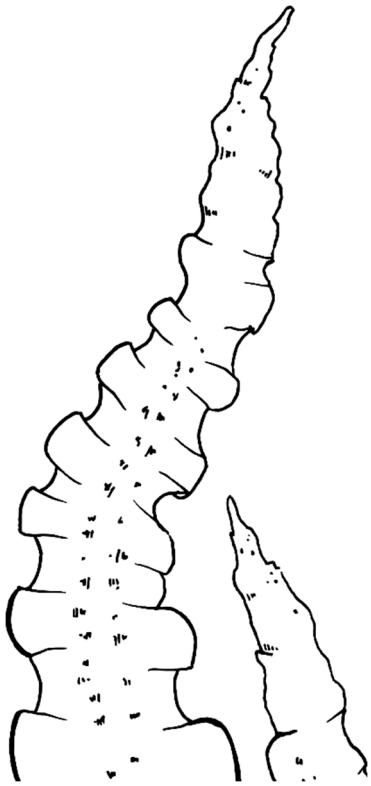
Doctor of natural sciences (Dr. rer. nat.)

Faculty 02 – Biology and Chemistry

University of Bremen, Germany

April 2021





Thesis reviewers

First reviewer: Prof. Dr. Kai Bischof
Marine Botany, University of Bremen
Bremen, Germany

Second reviewer: Prof. Dr. Ulf Karsten
Applied Ecology & Phycology, University of Rostock
Rostock, Germany

Thesis Colloquium: 20th May 2021

This research has been conducted at the Department of Marine Botany, Bremen Marine Ecology Centre for Research and Education (BreMarE), Faculty Biology/Chemistry, University of Bremen (Bremen, Germany). Experiments were executed at the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (Bremerhaven, Germany) and at the AWIPEV Research Station in Ny-Ålesund (Svalbard, Norway).

The study was further supported by Kellner & Stoll – Stiftung für Klima und Umwelt and by the 2015–2016 BiodivERsA COFUND call for research proposals (program MARFOR), with the national funder German Research Foundation (DFG; grant no. VA 105/25-1).

ALFRED-WEGENER-INSTITUT
HELMHOLTZ-ZENTRUM FÜR POLAR- UND MEERESFORSCHUNG

biodiversa

KELLNER & STOLL
STIFTUNG
FÜR KLIMA UND UMWELT
TREUHANDSTIFTUNG DER STIFTUNG DER UNIVERSITÄT BREMEN

***Saccharina latissima* in Kongsfjorden.**
© Sarina Niedzwiedz

"Never has it been more important to understand how the natural world works – and how to help it."

Sir David Attenborough
Our Planet

Table of Contents

Summary

In coastal ecosystems, large brown macroalgae are important foundation species, growing on rocky shores from temperate to polar regions. They form marine forests, also known as kelp forests, which are among the most productive coastal marine ecosystems in the world. Kelps – in the strict sense only including species within the order of the Laminariales – are of high ecological and economic value. For instance, they function as natural coastal protection, provide habitat for many organisms, and contribute to carbon sequestration. However, they also have high commercial benefits regarding aquaculture and biochemical compounds. Climate change poses a great threat to marine forests, as macroalgal community composition and distribution are largely governed by temperature. Thus, warming has vast implications on marine forests and their global biogeographic patterns. Importantly, also other drivers affect kelps and strong interactive effects are observed. The aim of this study was to gain a deeper understanding of the inter- and intraspecific acclimation processes of kelp species to abiotic conditions along large spatial and environmental gradients.

The thermal tolerance of the broadly distributed kelp *Saccharina latissima* towards marine summer heatwaves across latitudes was investigated (**publication I**). Field sporophytes were collected from five locations with different temperature regimes along the North East Atlantic coast and exposed to simulated heatwaves, by applying the respective mean summer temperature (Δ±0 °C) and enhanced temperatures of Δ+2 – 6 °C. Pronounced site-specific variability of the response patterns implied high phenotypic plasticity in European *S. latissima*. Moreover, the impact of realistic summer temperature increases did not differ based on potential temperature ecotypes. Exclusively isolates from warmer regions were impaired by simulated marine heatwaves, while samples from the central and expanding-edge populations exhibited rather neutral responses. Still, the successive increase in temperature in the experiment allowed specimens collected from rear-edge populations to survive considerably higher temperatures as reported in prior studies.

To reveal seasonal and inter-annual differences in the susceptibility of *S. latissima* towards marine heatwaves in summer, field sporophytes from Helgoland were collected in August 2018 and 2019 (**publication II**). The samples were exposed to the same temperature treatments as the individuals from Helgoland in **publication I** and results were compared to June 2018. Additionally, local sea surface temperatures between April and September 2018 and 2019 were analyzed. The upper temperature tolerance of *S. latissima* differed between seasons and years, and variation in the temperature history throughout the year was found to

alter the thermal susceptibility of the species. Consequently, warmer years and intensive marine heatwaves increase kelp mortality.

In the Arctic, increasing temperature is often followed by increased meltwater and nutrient input. Therefore, the impact of potential interactions of marine heatwaves in summer paired with hyposalinity or enhanced nutrient availability was investigated on *S. latissima* (**publication III**). Although co-existing drivers had been reported to affect synergistically or antagonistically resilience, this was not the case in Arctic field sporophytes of *S. latissima*. The applied realistic fluctuations in abiotic drivers neither compromised nor significantly promoted the specimens' physiological and biochemical status. Yet, enhanced nutrient conditions might support growth and vitality of *S. latissima* populations in Arctic habitats to a certain extent.

Publication IV focused on the biochemical and morphological variability of *S. latissima* across its entire distribution range in Europe. Sporophytes were collected from 16 different locations along the European Atlantic coast and the Baltic Sea, and links between morphology, biochemical composition, and genetic diversity were investigated. Dependency of morphological and biochemical traits on local abiotic conditions was detected, however, no distinct attribution to the geographical origin could be determined in the phenotypical profiles and genetics of European *S. latissima.* This indicates that 1) the impact of ambient abiotic conditions is diversified and complex, and 2) the species has not reached equilibrium levels in genetic differentiation yet and, thus, is in an ongoing process of intraspecific separation.

The combined effect of different temperatures and salinities on physiological and biochemical response variables was also investigated in young *Laminaria solidungula* sporophytes (**publication V**). The resilience of this Arctic endemic kelp species was affected – both positively and negatively – by the complex interaction of temperature increase and hyposalinity, impeding future prognoses of its responses towards climate change.

In conclusion, this thesis presents important information on the functional variability of two kelp species with different distributional ranges in Europe. Both studied species exhibit different sensitivity towards drivers related to environmental change. Furthermore, intraspecific variation must not be underestimated. The observed changes in the kelp populations of Europe, thus, cannot exclusively be ascribed to temperature variation but to an interplay of various abiotic and biotic factors. These findings contribute further knowledge to support future approaches to the conservation of marine forests.

Zusammenfassung

Entlang der Felsküsten gemäßigter und polarer Breiten wachsen große Braunalgen in sogenannten Kelpwäldern und formen hier eines der wichtigsten marinen Küstenökosysteme der Welt. Kelp – im engeren Sinne nur Arten der Ordnung Laminariales – sind von großer ökologischer sowie ökonomischer Bedeutung, da sie beispielsweise als natürlicher Küstenschutz fungieren, Habitate für viele Organismen schaffen und zur globalen Kohlenstoffbindung beitragen. Des Weiteren haben sie einen großen kommerziellen Wert für die Aquakultur und durch die Nutzung ihrer biochemischen Inhaltsstoffe. Die geographische Verteilung und Diversität von Makroalgenlebensgemeinschaften wird vor allem durch Temperatur gesteuert. Somit sind Kelpwälder durch Klimawandel und insbesondere durch den damit einhergehenden Temperaturanstieg stark betroffen. Zusätzlich wirken auch weitere Faktoren auf Kelp ein, wobei vielfältige Interaktionen verschiedener Faktoren beobachtet wurden. Die vorgelegte Studie zielte darauf ab, inter- und intraspezifische Anpassungsmechanismen von Kelparten an verschiedene Umweltverhältnisse entlang großer räumlicher und ökologischer Gradienten besser zu verstehen.

Die weitverbreitete Kelpart *Saccharina latissima* wurde auf ihre Temperaturtoleranz gegenüber marinen Hitzewellen entlang des latitudinalen Gradienten untersucht (**Publikation I**). An fünf Probenahmestandorten entlang der Küste des Nord-Ost-Atlantiks wurden Sporophyten gesammelt und diese anschließend simulierten Hitzewellen ausgesetzt, bei denen die jeweilige Durchschnittstemperatur im Sommer (Δ±0 °C) um Δ+2 bis Δ+6 °C erhöht wurde. Ausgeprägte ortspezifische Unterschiede innerhalb der gemessenen Parameter deuten auf eine hohe phänotypische Plastizität in *S. latissima* hin. Außerdem wurde nachgewiesen, dass der Einfluss realistischer Temperaturerhöhungen im Sommer sich aufgrund potentieller Temperaturökotypen nicht unterscheidet. Ausschließlich Individuen aus wärmeren Gebieten wiesen Beeinträchtigungen durch die simulierten Hitzewellen auf, wohingegen Proben aus zentralen und nördlichen Populationen neutral reagierten. Der schrittweise Temperaturanstieg während des Versuchs ermöglichte es den Exemplaren aus südlichen Randpopulationen jedoch, erheblich höhere Temperaturen zu überdauern als in vorherigen Studien beschrieben wurde.

Zur Aufklärung saisonaler und zwischenjährlicher Unterschiede in der Anfälligkeit von *S. latissima* gegenüber marinen Hitzewellen im Sommer wurden Sporophyten im August 2018 und 2019 rund um Helgoland gesammelt (**Publikation II**). Diese Proben wurden der gleichen

Temperaturbehandlung wie die Individuen aus Helgoland in **Publikation I** ausgesetzt und mit den Proben vom Juni 2018 verglichen. Zusätzlich wurden die lokalen Oberflächenwassertemperaturen zwischen April und September in den Jahren 2018 und 2019 ausgewertet. Die obere Temperaturtoleranz von *S. latissima* unterschied sich sowohl zwischen den Jahreszeiten als auch zwischen den Jahren. Diese Unterschiede basierten auf Schwankungen der Temperaturen im Jahresverlauf, welche die allgemeine Temperaturempfindlichkeit der Art veränderten. Folglich ist die Kelpsterblichkeit in wärmeren Jahren und nach intensiven Hitzewellen erhöht.

In der Arktis sind marine Hitzewellen im Sommer nicht nur mit Temperaturanstieg, sondern auch mit erhöhtem Schmelzwasser- und Nährstoffeintrag verbunden. Um mögliche Interaktionen zwischen den Parametern zu ermitteln, wurden arktische *S. latissima* mit verringerter Salinität oder erhöhter Nährstoffverfügbarkeit in Kombination mit Temperaturanstieg inkubiert (**Publikation III**). Obwohl verschiedentlich berichtet wurde, dass gleichzeitig auftretende Faktoren möglicherweise die Widerstandsfähigkeit synergistisch oder antagonistisch beeinflussen, konnte dies hier bei adulten arktischen *S. latissima* nicht nachgewiesen werden. Die Sporophyten waren nach realistischen Veränderungen der abiotischen Umwelteinflüsse weder physiologisch noch biochemisch beeinträchtigt. Erhöhte Nährstoffkonzentrationen könnten allerdings das Wachstum und die Vitalität arktischer Populationen von *S. latissima* geringfügig verbessern.

Die morphologische und biochemische Vielfalt von *S. latissima* über ihr gesamtes Verbreitungsgebiet in Europa wurde in **Publikation IV** untersucht. Sporophyten wurden an 16 verschiedenen Probenahmestellen entlang der europäischen Atlantikküste und der Ostsee gesammelt und Zusammenhänge zwischen Morphologie, biochemischer Zusammensetzung und genetischer Variabilität untersucht. Morphologische sowie biochemische Merkmale von *S. latissima* wurden nachweislich von lokalen abiotischen Faktoren beeinflusst, jedoch konnten weder die phänotypischen Profile noch die genetische Differenzierung präzise der geographischen Herkunft in Europa zugeordnet werden. Daraus resultiert, dass 1) der Einfluss der örtlichen Umwelteinflüsse vielfältig und komplex ist und 2) die Art noch kein fixiertes Äquilibrium in der genetischen Differenzierung erreicht hat und sich folglich in einem noch fortlaufenden innerartlichen Separierungsprozess befindet.

Der Einfluss der Wechselwirkung verschiedener Temperaturen und Salzgehalte auf physiologische und biochemische Parameter wurde auch an jungen Sporophyten von *Laminaria so-*

lidungula untersucht (**Publikation V**). Die Widerstandsfähigkeit dieser in der Arktis endemischen Kelpart wurde durch die komplexen Einwirkungen von Temperaturanstieg und Hyposalinität sowohl positiv als auch negativ beeinflusst. Dies erschwert zukünftige Prognosen ihrer Reaktionen auf den Klimawandel.

Die vorgelegte Dissertation fasst wichtige Erkenntnisse zur funktionellen Variabilität von zwei Kelparten unterschiedlicher Verbreitung in Europa zusammen. Die beiden untersuchten Arten weisen unterschiedliche Empfindlichkeit gegenüber schwankenden Umwelteinflüssen auf, und auch intraspezifische Unterschiedene dürfen nicht unterschätzt werden. Die schon zu beobachteten Veränderungen in Kelppopulationen in Europa können also nicht ausschließlich auf Temperaturschwankungen zurückgeführt werden, sondern beruhen auf Interaktionen verschiedener abiotischer und biotischer Faktoren. Diese Erkenntnisse sind wichtiger Bestandteil für zukünftige Ansätze im Umweltschutz zur Erhaltung von Kelpwäldern.

Abbreviations

A	Antheraxanthin
Acc	Accessory pigment pool
ANOVA	Analysis of variance
bp	Base pair
C	Carbon
Car	Carotene
C:N ratio	Carbon to nitrogen ratio
Chl	Chlorophyll
CO_2	Carbon dioxide
COI	Cytochrome-*c*-oxidase I
DNA	Deoxyribonucleic acid
DPS	De-epoxidation of the xanthophyll cycle
DW	Dry weight
F_v/F_m	Optimum / Maximum quantum yield of photosystem II
Fuc	Fucoxanthin
GLM	Generalized linear models
HPLC	High performance liquid chromatography
LD	Light:dark
MHW	Marine heatwave
N	Nitrogen
NO_3^-	Nitrate
PAM	Pulse amplitude modulated fluorometer
PAR	Photosynthetically active radiation
PCA	Principal component analysis
PCR	Polymerase chain reactions
PES	Provasoli enriched seawater
PO_4^{3-}	Phosphate
ROS	Reactive oxygen species
S_A	Absolute salinity
SD	Standard deviation
SST	Sea surface temperature
SW	Seawater

SW+N+P	Seawater + nitrate + phosphate
UV radiation	Ultraviolet radiation
V	Violaxanthin
VAZ	Xanthophyll pigment pool
Z	Zeaxanthin
Δ	Delta

1. General Introduction

1.1. Marine forests

Marine forests, also known as kelp forests, are among the most productive coastal marine ecosystems, characterized by canopy-forming large brown seaweed species (Phaeophyceae) of the orders Laminariales, Fucales and Tilopteridales (Teagle et al. 2017, Wernberg & Filbee-Dexter 2019). They are found on rocky shores from temperate to polar regions and are distributed along about 25 % of the world's coastlines (Krumhansl et al. 2016, Smale 2020). Even though the term 'kelp' is commonly used for almost any large, brown macroalga, in the strict taxonomical sense, it refers exclusively to species within the order of Laminariales (Dayton 1985, Fraser 2012). Kelp species are among the largest seaweeds in the world and their three-dimensional structures alter physical, chemical and biological conditions in the environment (Krause-Jensen & Duarte 2014, Teagle et al. 2017, Pfister et al. 2019, Wernberg et al. 2019). As ecosystem engineers, kelps provide food, shelter and nursery grounds for a huge variety of associated organisms (Christie et al. 2009, Krause-Jensen & Duarte 2014, Bennett et al. 2016) (see **Fig. 1.1**) but also act as a natural coastal defense by modifying water velocity or causing wave damping (Løvås & Tørum 2001).

Fig. 1.1: Marine forests in Europe. The three-dimensional structure of kelps, such as *Laminaria hyperborea*, provides a habitat for many associated organisms. Left: ©Uli Kunz, Submaris. Right: ©Calum Duncan, Marine Conservation Society.

Kelps greatly contribute to the global carbon cycle. They are among the most productive primary producers in the world (Mann 1973, Wernberg et al. 2019) and also act as an important carbon sink (Krause-Jensen & Duarte 2016). Not only does the appearance of kelp forests resemble their terrestrial counterparts, but also their primary productivity rivals temperate and tropical forests (Lüning 1990, Filbee-Dexter 2020). The capacity of seaweeds as carbon sink eventually supports mitigation of climate change since about 10 % of their global net

primary production is estimated to be sequestered (Krause-Jensen & Duarte 2016). Just recently, kelp biomass was reported to be extensively exported to the deep sea (3,000 – 4,000 m) mainly as particulate organic matter and thereby is blocked from exchanging with the atmosphere and, resulting in carbon burial over large timescales (Ortega et al. 2019). Consequently, there are auspicious approaches to bind anthropogenic CO_2 via macroalgae, so-called 'blue carbon' strategies (Nellemann et al. 2009, Chung et al. 2011, Duarte et al. 2017, Froehlich et al. 2019).

However, 'blue carbon' is just one part representing the high value of ecosystem services provided by kelp forest ecosystems. Besides their important ecological function, kelps are of high socio-economic relevance. Humans already benefitted from kelps several thousand years ago (Erlandson et al. 2007, Dillehay et al. 2008). Historically, they were used as food and medicine, and today their utilization is manifold (Dillehay et al. 2008, Kim et al. 2017). Pigments, lipids, phenolics and polysaccharides, and other biochemical compounds serve commercially as e.g. fertilizers, animal feed, biofuels, cosmetics or pharmaceuticals (Vásquez 2008, Stengel et al. 2011, Hafting et al. 2015, Camus et al. 2016). Kelp aquacultures and wild harvesting globally exceeded more than 8 million tons with a value of about US$ 1.4 billion in 2014, and kelp production in western countries has become one of the fastest-growing industries (Kim et al. 2017). In Europe, the majority (99 %) of the harvested seaweed biomass is from wild stocks (Mac Monagail et al. 2017). Kelp harvesting in Norway accounts for > 50 % of European macroalgal biomass (Stévant et al. 2017). However, not only the products of kelps are of great value. Kelp forest ecosystems also contribute to the economy by providing jobs in commercial fishing, retail and reef-related tourism (Bennett et al. 2016). The 'Great Southern Reef' along the southern coast of Australia alone generates AU$ 10 billion per year only by fishing and tourism (Bennett et al. 2016). Overall, the total value of kelp forests has been currently estimated at US$ 0.5 – 1 million per year and per kilometer of coastline (Filbee-Dexter & Wernberg 2018). Consequently, changes in the global abundance of kelp forests have far-reaching impacts on ecosystem services and economic value.

1.2. Climate change – The impact of ocean warming and other drivers

Global climate change has profound implications on all ecosystems on the planet (Parmesan 2006) since environmental changes compromise the survival of species and the associated ecosystem functions (Harley et al. 2012). In this context, particularly increasing temperatures were reported to be a great threat to coastal marine ecosystems (Harley et al. 2006, Pörtner & Farrell 2008, IPCC 2019, Smale 2020). Global sea surface temperature (SST) has increased by > 0.6 °C since the mid-19th century and current models predict it will further increase by at least 1.6 – 4 °C until the end of the 21st century (IPCC 2019). The North East Atlantic is one of the hotspots of global warming, where SST has risen between 0.3 – 0.8 °C per decade since 1980 (Lima & Wethey 2012). In addition to long-term ocean warming, marine heatwave (MHW) events are projected to increase in intensity and frequency (Frölicher et al. 2018). MHWs describe short-term increases of SST by 3 – 5 °C above the long-term mean (Meehl & Tebaldi 2004, Hobday et al. 2016). Between 1925 and 2016, the annual duration of MHWs has already increased by 54 % worldwide (Oliver et al. 2018).

Temperature is a major factor controlling global biogeographic patterns of marine benthic algal communities. Thus, changes in temperature are driving redistribution of species on global scales (Lüning 1990, Adey & Steneck 2001, Wernberg et al. 2015, Harris et al. 2018, Smale 2020). In the last five decades, marine forests have undergone big changes in abundance and distribution. Krumhansl et al. (2016) reported a decline in kelp populations in 38 % of the examined regions but also an increase or stabilization of communities in 68 %. The impact of ocean warming and MHWs on kelps and other foundation seaweeds are well studied (e.g. Bennett et al. 2015, Burdett et al. 2019, Nepper-Davidsen et al. 2019, Saha et al. 2020, Supratya et al. 2020), and declines of marine forests have been reported from all over the world (e.g. Bekkby & Moy 2011, Voerman et al. 2013, Filbee-Dexter et al. 2019, 2020, Thomsen et al. 2019, Wernberg et al. 2019). Until 2100, further shifts and extinctions of different seaweed populations were projected in model studies (Müller et al. 2009, Assis et al. 2018). The loss of marine forests is often followed by an ecosystem shift to ephemeral, filamentous turf algae communities (Eriksson et al. 2002, Moy & Christie 2012, Filbee-Dexter & Wernberg 2018, Christie et al. 2019a).

However, not only temperature influences the distribution of marine forests. The variation in many other abiotic drivers, both at a global and local scale, greatly affects kelp species on physiological and biochemical level. Ocean acidification caused by increased CO_2 levels in the atmosphere (Olischläger et al. 2012, Roleda et al. 2012, Fernández et al. 2015, Britton et al.

2016) and changes in UV radiation due to ozone depletion or seasons (Hanelt et al. 2001, Bischof et al. 2002) have an impact on large scales, whereas variations in irradiance throughout the water column (Bischof et al. 1998, Wiencke et al. 2006, Laeseke et al. 2019), eutrophication after increased nutrient input (Gerard 1997, Pfister & Van Alstyne 2003, Gordillo et al. 2006, Fernández et al. 2020) or fluctuations in salinity (Peteiro & Sánchez 2012, Springer et al. 2017, Li et al. 2020, Monteiro et al. 2021) occur locally and affect kelp populations on smaller scales.

Further threats impairing marine forests are, for instance, overgrazing by sea urchins (Hart & Scheibling 1988, Filbee-Dexter & Scheibling 2014), storm-generated destructions and sediment deposition (Seymour et al. 1989, Gaylord et al. 2008, Roleda & Dethleff 2011), turbidity resulting from increased sedimentation and particle load (Bischof et al. 2019) or commercial harvesting (Christie et al. 1998, 2009).

Strong interactive effects between different drivers were observed, revealing both synergistic and antagonistic impacts on kelps (e.g. Roleda et al. 2008, Roleda 2009, Rothäusler et al. 2011, Heinrich et al. 2015, Martins et al. 2017, Olischläger et al. 2017, King et al. 2018b, Li et al. 2020). Additionally, the impact of climate change is not uniform around the globe and intraspecific local adaptation across latitudes can result in site-specific responses to abiotic stressors (King et al. 2018a, IPCC 2019). Thus, it is of great importance to consider the diversity and complexity of the different drivers to enable reliable prognoses on future developments of marine forests and subsequent conservation strategies.

1.2.1. Kongsfjorden – A natural laboratory in the Arctic

The strongest regional warming over the last 30 years was detected in the polar regions, where the temperature in the atmosphere increased more than twice as fast as the global average (Maturilli et al. 2013, Meredith et al. 2019), with far-reaching consequences for Arctic marine ecosystems.

Elevated air and ocean temperatures result in the melting of glaciers and sea ice, and in an increased terrestrial freshwater run-off from snowfields (Sundfjord et al. 2017, Filbee-Dexter et al. 2019). The total freshwater inflow into the Arctic Ocean increased by ~ 7 % from 1936 to 1999 (Bluhm & Gradinger 2008) and by 40 % from 2003 to 2007 in the Beaufort Gyre (Meredith et al. 2019). Further warming and an increased frequency of heatwave events were predicted for the long term, likely resulting in accelerated reduction of ice coverage in the

Arctic (Müller et al. 2009, Walczowski et al. 2012, Oliver et al. 2018). The consequences of glacial and terrestrial discharge are increased sediment load in coastal environments, variations in nutrient availability, and the transient generation of hyposaline conditions in fjord systems. The latter has been explained by limited seawater exchange and stratification (Svendsen et al. 2002, Spurkland & Iken 2011a, Krause-Jensen et al. 2012, Bischof et al. 2019). Kongsfjorden, on the west coast of Svalbard, is particularly affected by warm off-shore Atlantic waters and is considered to be a 'hot spot' of climate change in polar regions (Sundfjord et al. 2017). This notion, together with its scientific infrastructure and year-round accessibility, makes Kongsfjorden an ideal model system for studying climate change impacts on Arctic fjord systems (Bischof et al. 2019). Current mean winter air temperatures have increased by at least 8 °C compared to 20 years ago (Maturilli et al. 2013). In the period from 1996 to 2000, mean summer SST of 4 °C were measured (Svendsen et al. 2002), between 2006 to 2011 the SST exceeded 6 °C (Dalpadado et al. 2016), and SST maxima of almost 8 °C were measured in summer 2019 (Alfred Wegener Institute 2019). In Kongsfjorden, the surface salinity frequently drops from S_A 34 to 28 during summer (Svendsen et al. 2002). However, temporary freshwater inflow can result in hyposaline conditions of $S_A < 23$ down to 20 m water depth, after vertical mixing by wave and wind action (Hanelt et al. 2001, Karsten et al. 2003, Karsten 2007). The increasing air and water temperatures have frequently precluded winter sea-ice formation in the fjord (Cottier et al. 2005, 2007). In contrast to Antarctic regions, strong seasonality in nutrient availability was monitored in Arctic waters (Aguilera et al. 2002, Bischof et al. 2019). Nutrient concentrations in Kongsfjorden are relatively high in winter and early spring (NO_3^-: 7 – 10 µM, PO_4^{3-}: 0.5 – 0.7 µM). This is followed by nutrient depletion between spring and fall (NO_3^-: < 0.05 µM, PO_4^{3-}: ~ 0.1 µM) as a result of phytoplankton blooms, which occur as soon as enough light is available and waters are ice-free (Rokkan Iversen & Seuthe 2011, Bischof et al. 2019). At Kongsfjorden, the polar night lasts from the end of October to mid-February (Bischof et al. 2019). The decline of sea ice enables light to transmit earlier, deeper, and with higher irradiance into the water column, resulting in an earlier start of the spring season which affects all phototrophic organisms, i.e. also seaweeds (Krause-Jensen et al. 2012, Bischof et al. 2019). Increases in biomass of kelps and decrease in their depth extension have already been reported for Kongsfjorden (Bartsch et al. 2016), and further changes in benthic community structure are expected with progressing changes in the environment (Müller et al. 2009, Krause-Jensen & Duarte 2014).

1.3. Kelp along the European coast

The order Laminariales, including 105 species in 30 genera, is distributed along the coasts of the entire Northern Hemisphere (Bolton 2010). It originated in the northern Pacific about 25 million years ago, before its species crossed the Bering Strait ~ 5.3 million years ago (Bolton 2010, Rothman et al. 2017). Kelp species are widespread throughout temperate and polar regions of the North Atlantic coast, where they grow at subtidal shallow rocky coasts down to 30 m (Araújo et al. 2016). The European coastline is dominated by the genera *Alaria*, *Laminaria* and *Saccharina* (Bolton 2010, Araújo et al. 2016).

Kelps are characterized by a haplo-diplotic heteromorphic life cycle (Hurd et al. 2014) with microscopic, haploid (n) gametophytes and macroscopic, diploid (2n) sporophytes (**Fig. 1.2**). The present study, however, only focuses on kelp sporophytes. Sporophyte thalli are complex and divided into holdfast (rhizoid), stipe (cauloid) and blade (phylloid, lamina) (van den Hoek et al. 1995).

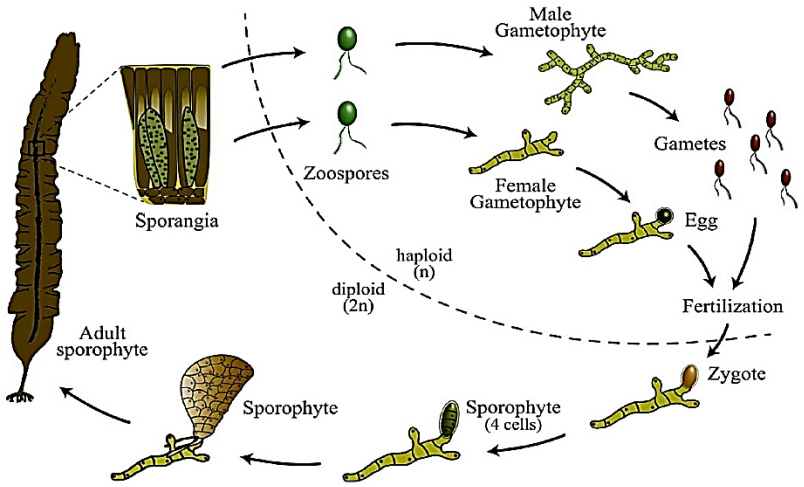


Fig. 1.2: Schematic illustration of the life cycle of kelps (Visch et al. 2019). *Note*: Not at scale; gametophytes are in the range of a few millimeters, while sporophytes can grow up to a size of several meters.

1.3.1. *Saccharina latissima*

The sugar kelp *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (Lane et al. 2006), formerly known as *Laminaria saccharina*, is a boreal-temperate species with a wide latitudinal range in the Northern Hemisphere, reaching from polar to temperate regions (Bolton & Lüning 1982, Araújo et al. 2016). Even though the Iberian Coast at ~ 40°N is the southernmost distribution of *S. latissima* in Europe, it does not necessarily represent the warmest region of occurrence due to frequent upwelling of cold deep water (Lourenço et al. 2016). The highest sea surface temperatures are regularly measured in southern Brittany and around Helgoland (Giovanni Satellite: Acker & Leptoukh 2007). Stable populations of *S. latissima* are monitored in the Baltic Sea (Araújo et al. 2016), where absolute salinity (S_A) strongly declines to around S_A 10 in the southwest (Kautsky & Kautsky 2000). Pronounced separation in distinct populations with a high degree of inter-population differentiation but no significant variation in genetic diversity with latitude was determined for *S. latissima* from Europe (Guzinski et al. 2016, 2020, Nielsen et al. 2016, Luttikhuizen et al. 2018).

Fig. 1.3: Left: Sporophyte of *Saccharina latissima*. Illustration by William H. Harvey (1846). Right: Adult sporophytes collected nearby Locmariaquer (Brittany, France). The white scale represents 2 m. Photo by Nora Diehl.

Saccharina latissima grows on rocky shores in the upper subtidal to depths of 15 – 30 m (Wiencke et al. 2004, Pehlke & Bartsch 2008, Bekkby & Moy 2011, Bischof et al. 2019) and prefers sheltered conditions (Lüning 1990). The dominant sporophyte (**Fig. 1.3**) typically forms narrow fronds and solid cauloids under medium wave exposure, possessing basal meristematic growth (Lüning 1990, van den Hoek et al. 1995). The phylloid is elongated, undivided, without a midrib but with a wrinkled surface (bullations) and wavy margins (White & Marshall 2007). The adult sporophyte can reach lengths of up to 4 m and attaches to hard substrate with a branching claw-like holdfast (White & Marshall 2007).

Overall, the species tolerates strong variations in environmental conditions. It survives temperatures from 0 – 23 °C for shorter periods, but mortality rate highly increases > 20 °C (Fortes & Lüning 1980, Bolton & Lüning 1982, Lüning 1984, 1990). Gametophytes of Laminariales were reported to generally exhibit a higher upper thermal tolerance of 3 – 4 °C above their sporophytes (tom Dieck 1993). The optimum growth temperature of sporophytes is between 10 and 15 °C (Bolton & Lüning 1982). With regard to salinity, the highest growth activity of *S. latissima* has been recorded between S_A 23 and 31, with a strong reduction in growth below S_A 16 and a strongly increasing mortality at S_A < 8 (Gerard et al. 1987, Karsten 2007). Another recent work, studying *S. latissima* in the context of cultivation methods, however, has reported optimum growth at salinities up to S_A 35 and at temperatures down to 5 °C (Kerrison et al. 2015). *Saccharina latissima* tolerates oligotrophic conditions, but their optimum nitrate concentration is around 10 µM (Chapman et al. 1978, Bartsch et al. 2008, Kerrison et al. 2015). Massive alterations – both expansions and declines – regarding abundance and depth distribution of *S. latissima* populations have been reported from all over Europe (Sweden: Eriksson et al. 2002, Helgoland: Pehlke & Bartsch 2008, Norway: Bekkby & Moy 2011, Moy & Christie 2012, Iberian Coast: Casado-Amezúa et al. 2019). In Kongsfjorden (Spitsbergen, Norway), *S. latissima* also experienced a slight increase in biomass and a slight reduction in depth extension between 1996/1998 and 2012/2013 (Bartsch et al. 2016). Species distribution models project an ongoing northward shift in the entire distributional range of *S. latissima* until 2100 with consequential disappearance at the southern distribution boundary and further expansion into the Arctic (Müller et al. 2009, Assis et al. 2018, Westmeijer et al. 2019).

1.3.2. *Laminaria solidungula*

Laminaria solidungula J. Agardh is the only truly endemic Arctic kelp species, growing in the Canadian, Alaskan and Russian High Arctic and along the coasts of Greenland and Svalbard at latitudes > 75°N (Lüning 1990, Belseth 2012, Wilce & Dunton 2014, Filbee-Dexter et al. 2019). It is found on hard substrate in the lower sublittoral zone at depths of 2 – 20 m (Wiencke et al. 2004). The sporophytes (**Fig. 1.4**) have a single blade with annual bands, which are described as heart- or circular-shaped and which represent the gain in the phylloid area during one growth season (Lüning 1990, Belseth 2012). Individuals can grow up to 2.5 m in length but are normally about 1 m long.

Gametophytes of *L. solidungula* survive temperatures between −1.5 and 20 °C for shorter periods, while sporophytes have a lethal temperature limit of 16 °C and optimum growth between 5 and 10 °C (tom Dieck [Bartsch] 1992, tom Dieck 1993). The species is extremely well adapted to low solar irradiation and can withstand long periods of total darkness (Dunton et al. 1982, tom Dieck 1993, Henley & Dunton 1997, Roleda 2016) by respiring its storage carbohydrates (Dunton & Schell 1986, Henley & Dunton 1995, Scheschonk et al. 2019). As a so-called 'season anticipator' *L. solidungula* predominantly grows in winter and early spring, while 'season responders', such as *S. latissima*, exhibit the highest growth activity when irradiance and temperature are optimal (Chapman & Lindley 1980, Dunton 1985, Kain [Jones] 1989, Wiencke et al. 2009). During the short light period in the Arctic, *L. solidungula* fixes carbon which is necessary for metabolism, growth and reproduction (Chapman & Lindley 1980, Dunton et al. 1982). It is also characterized as a 'storage specialist' for nitrate uptake (Korb & Gerard 2000). Thereby, *L. solidungula* can prevent nutrient competition with other phototrophic organisms (Bartsch et al. 2008). Comparable to *S. latissima,* the species can be considered stenohaline (Karsten 2007). Populations of *L. solidungula* are projected to extend further northward until the end of this century and to retreat at their southern distributional range, i.e. disappearing from southern Spitsbergen (Müller et al. 2009, Assis et al. 2018).

Fig. 1.4: Left: Sporophytes of *Laminaria solidungula* with the typical heart-shaped blades (Filbee-Dexter et al. 2019). Right: *L. solidungula* population, with associated biota (Wilce & Dunton 2014).

1.4. Adaptation and acclimation of kelp

1.4.1. Definitions

Generally, species respond to changes in the environment through migration, acclimation or adaptation, although these adjustments are not mutually exclusive.

Acclimation = fast and reversible (short-term) physiological adjustments of an individual in response to transitory changes in environmental conditions; expression of phenotypic plasticity (Leroi et al. 1994, Morgan-Kiss et al. 2006).

Adaptation = process of genetic changes (long-term) that occurs over many generations in response to an organism's specific environmental niche (Morgan-Kiss et al. 2006).

Adaptive plasticity = phenotypic plasticity that increases the global fitness of a genotype (Nicotra et al. 2010).

Ecotype = locally adapted populations that are phenotypically and genetically differentiated for adaptive traits; emerged by long-term exposure to different selective environmental pressures (Conner & Hartl 2004, Nicotra et al. 2010).

Fitness = the ability of an organism – or population – to survive and reproduce in its prevailing environment (Holderegger et al. 2006, Orr 2009).

Genotype = the entire genomic sequence (genetic composition) of an organism (Churchill 1974, Nicotra et al. 2010).

Phenotype = outward appearance, including chemical, structural and behavioral traits of a genotype resulting from both genetic and environmental influences (Churchill 1974, Holde-regger et al. 2006, Nicotra et al. 2010).

Phenotypic plasticity = the ability of a single genotype to modify its phenotype in response to changing conditions; the range of phenotypes a genotype can express as a function of its environment; within-population plasticity (Nicotra et al. 2010, King et al. 2018a).

Rear edge = **trailing edge** = populations at the low-latitude limit of species distribution (Hampe & Petit 2005).

Expanding edge = **leading edge** = populations at the high-latitude limit of species distribution (Hampe & Petit 2005).

1.4.2. Response mechanisms to environmental conditions

As sessile organisms, kelps cannot escape local stressors and constantly need to cope with varying environmental circumstances. They adjust their growth activity to seasons, fertility stage and latitudinal distribution (e.g. Fortes & Lüning 1980, Lüning 1988, Sjøtun 1993) and acclimate their photosynthetic efficiency to maximize performance (Hurd et al. 2014). However, the supply of nitrogen is considered to be the limiting factor for macroalgal productivity, particularly in summer (Roleda & Hurd 2019). In Laminariales, significant reduction in growth, photosynthetic performance and pigment content are observed under nutrient depletion (Bartsch et al. 2008). In contrast, enhanced nutrient concentrations have supportive effects on kelps (Gerard 1997, Henley & Dunton 1997, Bischof et al. 1998, Bartsch et al. 2008, Gao et al. 2016, Fernández et al. 2020). The nutrient status in macroalgae can be surveyed by determining the carbon (C) to nitrogen (N) ratio (Atkinson & Smith 1983). Low C:N ratios (< 20) in the algal tissue represent sufficient N supply (Atkinson & Smith 1983, Wiencke & Bischof 2012). However, the C:N ratio can also deviate from available seawater nutrient concentration. Correlation with seasons as well as morphology has been reported for kelps (Henley & Dunton 1995, Gevaert et al. 2001, Peters et al. 2005). Other factors affecting nitrogen uptake are enzymatic activity, which varies with different environmental conditions, such as temperature and salinity (Gordillo et al. 2002, Mandal et al. 2015, Roleda & Hurd 2019). The total C content constitutes the amount of all carbohydrates, i.e. alginate, cellulose and other polysaccharides (Peters et al. 2005, Amsler 2008), and C assimilation and storage are interlinked with growth activity (Wiencke & Bischof 2012).

Kelps and other seaweeds have also developed a wide range of biochemical mechanisms to adjust to environmental changes, i.e. in response to light as the main trigger, kelps adjust their pigment content and composition (Wiencke & Bischof 2012, Hurd et al. 2014). Apart from providing energy to photosynthesis, irradiance, including UV radiation, can also be an intensive stressor and varies with water depth, latitude and season and impairing effects of excess irradiance on kelps were reported in many studies (e.g. Franklin & Forster 1997, Müller et al. 2012, Laeseke et al. 2019). For example, high light stress induces the formation of reactive oxygen species (ROS), which then causes intracellular strain (Dring 2006). As a protection measure, excessive energy is dissipated by (chemically) reducing violaxanthin to antheraxanthin and further to zeaxanthin (de-epoxidation state of the xanthophyll cycle; DPS) (Goss & Jakob 2010). Present ROS are scavenged by phlorotannins, which act as antioxidants and

therefore are important photoprotective compounds (Amsler 2008, Gómez et al. 2016). Moreover, phlorotannins are multifunctional polyphenols and are also involved in antifouling bioactivity, feeding deterrence and wound healing, among others (Amsler 2008, Stengel et al. 2011).

Salinity variation and desiccation lead to osmotic stress in seaweeds with consequences, e.g. on growth (Gerard et al. 1987, Lüning 1990), photosynthetic performance (Karsten 2007, Gylle et al. 2009) or nutrient uptake (Jiménez & Niell 1991, Gordillo et al. 2002). Additionally, changes in phenolic concentrations, phycobiliproteins (pigments) and general protein level were reported (Stengel et al. 2011). Short-term adjustment to salinity change can be achieved via ion transfer (Kirst 1989), but with increasing and prolonged osmotic pressure, seaweeds synthesize and accumulate organic osmolytes. These act as 'compatible solutes' (Brown & Simpson 1972) to conserve intracellular homeostasis and maintain cellular function (Kirst 1989). Phaeophyta synthesize the polyol mannitol, which is interconverted with the long-term C storage product laminarin (Yamaguchi et al. 1966, Kirst 1989, Iwamoto & Shiraiwa 2005, Karsten 2012, Graiff et al. 2016). Besides its function as an osmolyte, mannitol was also confirmed as an anti-freezing compound (Monteiro et al. 2021) and antioxidant (Shen et al. 1997, Jennings et al. 1998).

Temperature is considered to be the most important factor determining biogeographic distribution (Adey & Steneck 2001), ultimately by controlling the physiology and biochemical status of seaweeds. Thus, several diversified stress responses are applied by kelps to changes in temperature. Overall effects on metabolism and enzymatic activity, growth and photosynthesis are long known (Davison 1987, Davison & Davison 1987, Lüning 1990, Davison et al. 1991, Daniel et al. 1996). More recent studies have shown that temperature stress also triggers the formation of ROS (Zhou et al. 2010), and can result in structural weakening of kelp tissue (Simonson et al. 2015b), changes in pigment composition (Andersen et al. 2013, Fernandes et al. 2016) or C:N ratio (Peters et al. 2005).

Many studies have focused on the response patterns of kelps to one abiotic driver (e.g. Davison & Davison 1987, Pfister & Van Alstyne 2003, Karsten 2007, Simonson et al. 2015a, b, Nepper-Davidsen et al. 2019), while considerably fewer studies investigated the interactive effects of different abiotic or biotic drivers (Henley & Dunton 1997, Fredersdorf et al. 2009, Heinrich et al. 2015, Zacher et al. 2016, Endo et al. 2017, Martins et al. 2017, Provost et al. 2017, Christie et al. 2019b, Li et al. 2020, Monteiro et al. 2021). Even though multiple-factor approaches are of high ecological relevance, the impact of interacting factors on kelps still

remains understudied (Mineur et al. 2015). Interaction of different stressors can have both synergistic or antagonistic effects for organisms, or can even result in cross-acclimation to abiotic conditions (Leshem & Kuiper 1996, Lotze & Worm 2002, Moy & Christie 2012, Springer et al. 2017, Fernández et al. 2020). Therefore, a fundamental understanding of physiological and biochemical response mechanisms is important to assess how foundation species and ecosystem engineers will respond to climate change.

Species generally respond to environmental change by phenotypic plasticity or by adaptive modifications. Organisms thrive in distinct environmental ranges and exhibit different specific limits in survival, growth and reproduction (Lüning 1990, Sunday et al. 2015). Thereby, the temperature is of primary importance for benthic marine algae. In general, margin populations are more affected than range center populations (Thomas 2010, Mota et al. 2015, King et al. 2019), as for example shown for rear-edge populations, which often live close to their upper thermal limits (Hampe & Petit 2005). Once the temperature exceeds thermal tolerance, physiology and fitness are impaired and may result in increased mortality and, eventually, local extinction of populations (Hampe & Petit 2005, Bennett et al. 2015). Species with a wide distribution are considered more tolerant than narrow-ranging species (Wiencke et al. 1994, Kelly et al. 2012, Sunday et al. 2015). However, local adaptation and phenotypic plasticity can result in intraspecific variability in thermal tolerance and performance to temperature. Long-term exposure to different selective environmental pressures eventually causes the emergence of locally adapted ecotypes (Nicotra et al. 2010). Consequently, population loss might not only occur at distributional rear edges (Bartsch et al. 2013, King et al. 2018a).

1.5. Aim of the study

The overarching aim of this study is to gain a deeper understanding of the inter- and intraspecific acclimation processes of kelps to abiotic conditions on large spatial scales. The study focuses on the biochemical characterization of kelps and their physiological and biochemical response mechanisms to short-term temperature increases in summer and potential interactions with salinity or nutrients. *Saccharina latissima* (boreal-temperate) and *Laminaria solidungula* (Arctic endemic) were chosen as representatives to study responses in kelps with different distributional ranges. Especially, the investigation of *S. latissima* across its entire distribution range in Europe allows achieving a better understanding of the impact of respective local conditions on the stress tolerance and the biochemical profile of different populations. This study provides valuable information on the functional variability and dynamics of marine forests facing climate change. The obtained results will add to the knowledge required for reliable prediction of future distribution patterns of these two important kelp species in response to environmental changes and may allow for the design and adaptation of required conservation strategies.

1.6. Thesis outline and research questions

Temperature as the main factor controlling global biogeographic patterns of marine benthic algal communities has been studied for many decades (e.g. Bolton & Lüning 1982, Lüning 1984, 1990, Adey & Steneck 2001, Wiencke & Bischof 2012). Today, with the emerging understanding of the consequences of climate change, the research on marine heatwaves (MHWs) is of utmost significance (e.g. Frölicher et al. 2018, Oliver et al. 2018, 2021, Smale et al. 2019, Hayashida et al. 2020), and their general impact on kelps and other foundation species was studied in detail over the last years (e.g. Winters et al. 2011, Bennett et al. 2015, Burdett et al. 2019, Nepper-Davidsen et al. 2019, Thomsen et al. 2019, Filbee-Dexter et al. 2020, Saha et al. 2020).

Different seaweed populations are often regarded as one single homogeneous physiological unit, assuming that a single species exhibits similar thermal tolerances, independent of its growth site (Reed et al. 2011). Consequentially, populations at the range center are proposed to be less vulnerable than rear-edge populations, which already live in their upper thermal limit (Thomas 2010, Mota et al. 2015, King et al. 2019). Local adaptation and phenotypic plasticity eventually result in differentiation into thermal characteristics and fitness of different

populations (Reed et al. 2011, King et al. 2018a), although adaptive modifications and resulting ecotypes can be subtle (Liesner 2020, Martins et al. 2020). Models assuming uniform climatic envelopes may underestimate local acclimation and adaptations of broadly distributed species (Kelly et al. 2012, Filbee-Dexter et al. 2020). To date, only little is known about common acclimation responses of different seaweed populations along large latitudinal gradients (e.g. Pereira et al. 2015, Wernberg et al. 2016, Liesner et al. 2020). It is still unknown to what extent the respective local environmental conditions matter for the thermal tolerance and the biochemical characteristics of populations on large distributional scales.

Hypothesis I: **The geographical origin of *Saccharina latissima* is reflected in different phenotypes, and sporophytes exhibit habitat-specific signatures.**

Hypothesis II: **The seasonal thermal history determines the tolerance of *Saccharina latissima* to marine heatwaves.**

In this context, it was proposed that reduced population resilience contributes to kelp mortality after exposure to suboptimal temperatures (Wernberg et al. 2010, Andersen et al. 2013, Provost et al. 2017), but it is unknown to what extent different abiotic factors contribute to the thermal tolerances of different kelp species. As reported for the interdependency of hyposalinity and UV susceptibility in *Alaria esculenta* (Springer et al. 2017) and of nitrogen sufficiency and heat tolerance in *Macrocystis pyrifera* (Fernández et al. 2020), one stressor might in fact also improve the tolerance of the algae to a second stressor.

Since *S. latissima* is broadly distributed, it has to cope with great variability of diverse environmental conditions and potential interactive effects. Profound patterns with genetically distinct populations are reported for *S. latissima* within Europe (Guzinski et al. 2016, 2020). Northern populations at the expanding edge in the Arctic have to face, for example, very low temperatures and periods of complete darkness. Rear-edge populations at the low-latitudinal distribution limit, in contrast, are exposed to comparatively high temperatures and strong irradiance. Besides these large-scale patterns, anomalies in temperature, salinity and nutrients can also occur on smaller scales. In the Baltic Sea, for example, the alga experiences a significant decrease in salinity from West to East (Kautsky & Kautsky 2000), and along the Iberian Coast, upwelling events drastically change local conditions (Lourenço et al. 2016). Furthermore, *S. latissima* is exposed to long-term changes, such as ocean warming or ozone

depletion, and short-term events, e.g. MHWs, snow and sea ice melting (United Nations Environment Programme 2014, IPCC 2019). The widespread distribution and high degree of physiological responses and polymorphism (reviewed by Bartsch et al. 2008) speak for the existence of ecotypes, postulated in several studies (Lüning 1975, Gerard & Du Bois 1988, Müller et al. 2008, Spurkland & Iken 2012, Olischläger et al. 2014, 2017). However, whether *S. latissima* exhibits ecotypes is still not fully resolved as other studies did not find evidence for ecotypic differentiation and rather suggest high phenotypic plasticity (Bolton & Lüning 1982, Spurkland & Iken 2011b).

In comparison, much less is known about *Laminaria solidungula* and contrary to *S. latissima*, it only exhibits a very limited distribution range. As an endemic species, it has adapted perfectly to Arctic conditions regarding temperature, light availability and nutrients (tom Dieck 1993, Henley & Dunton 1997, Korb & Gerard 2000, Roleda 2016). Future northward expansion and southern extinction were proposed for *L. solidungula* (Müller et al. 2009, Assis et al. 2018). However, the effects of potential interactions of abiotic drivers are still poorly understood.

<u>Hypothesis III</u>: Hyposalinity and nutrient enrichment affect the thermal susceptibility of kelps from the Arctic.

In order to shed light on the complexity of adaptive responses to interacting abiotic drivers in kelps along large spatial and environmental gradients, the following research questions have been addressed:

Research question I:

Do populations of European *Saccharina latissima* reveal different thermal tolerances to marine heatwaves in summer across its latitudinal distribution gradient?

For **publication I**, meristematic discs of field sporophytes were collected in summer 2018, 2019 and 2020 from five different locations along the European Atlantic coast: Spitsbergen, Norway (79°N), Bodø, Norway (67°N), Bergen, Norway (60°N), Helgoland, Germany (54°N) and Locmariaquer, France (47°N). In the experimental set-up, each respective local mean summer temperature (control, Δ±0 °C) was increased up by Δ+2, Δ+4 and Δ+6 °C. Physiological performance was monitored by survival, growth and optimum quantum yield of the samples during the temperature-amplitude treatments. Mannitol, C:N, pigment content and phlorotannins were analyzed after the experiment to reveal local differentiation in biochemical stress responses of *S. latissima* to relative marine heatwave events in summer over a large geographical scale.

Research question II:

Do seasonal and inter-annual differences in thermal history affect stress responses of *Saccharina latissima* to marine heatwaves in summer?

For **publication II**, field sporophytes from Helgoland (54°N) collected in August 2018 and 2019 were treated exactly like the samples from Helgoland in **publication I**, applying absolute temperatures of 18, 20, 22 and 24 °C. Survival, growth, optimum quantum yield and pigment content were evaluated. Additionally, water temperatures (provided by COSYNA system) around Helgoland were analyzed from April – September 2018 and 2019. The stress responses in August 2018 were compared with the ones in June 2018 (see **publication I**) to reveal seasonal differences. For revealing inter-annual differences, results from August 2018 and August 2019 were compared.

Research question III:

Does the interaction of marine summer heatwaves and hyposalinity or nutrient enrichment affect the thermal susceptibility of Arctic *Saccharina latissima*?

For **publication III**, two short-term two-factorial experiments (temperature × salinity, temperature × nutrients) were conducted with field sporophytes from Kongsfjorden, Svalbard (N 78°55.50', E 11°55.11') collected in summer 2019. Temperature treatments for both experiments were set to 4, 6, 8 and 10 °C to mimic realistic Arctic marine heatwave scenarios in summer. The hyposalinity treatment was based on the minimum salinity measured in Kongsfjorden over the summer and nutrient treatments were based on the highest yearly concentrations (winter). Growth and optimum quantum yield were analyzed every second day to monitor the physiological performance of the samples. As biochemical parameters, mannitol, C:N, pigment content and phlorotannins were analyzed before and after the experiment to identify different acclimation processes and potential interacting effects of the abiotic factors.

Research question IV:

Do *Saccharina latissima* populations in Europe reveal distinct patterns regarding morphology and biochemical composition?

For **publication IV**, meristematic parts from sporophytes of *Saccharina latissima* were collected from 16 different locations in the Arctic, North Sea, North East Atlantic and Baltic Sea in the summer periods of 2018, 2019 and 2020. The intraspecific variability along a latitudinal and a salinity gradient was investigated by analyzing links between morphology (length, width), biochemical composition (mannitol, C:N, phlorotannins) and genetic diversity (COI haplotype network).

Research question V:

How does the Arctic endemic species *Laminaria solidungula* acclimate to interacting environmental stressors?

For **publication V**, young sporophytes were exposed to a short-term two-factorial stress experiment (temperature × salinity). The experimental temperatures (0, 5, 10, 15 °C) were based on the sporophytes' thermal survival range and hyposalinity on the minimum salinity frequently measured in Kongsfjorden, Svalbard. Sporophytes were raised from a gametophyte strain of *L. solidungula* (as stock culture: AWI culture number 3130) from Spitsbergen. Photosynthetic performance was monitored during the experiment. At the end of the experiment, mannitol, C:N, pigments and phlorotannins were analyzed to investigate acclimation processes to different temperatures and hyposalinity and to identify potential interaction between the two factors.

1.7. List of publications and declaration of contributions

Publication I:

Title: Summer heatwave impacts on the European kelp *Saccharina latissima* across its latitudinal distribution gradient

Authors: **Nora Diehl**, Michael Y. Roleda, Inka Bartsch, Ulf Karsten, Kai Bischof

Journal: Frontiers in Marine Science (submitted, April 2021)
Raw data: uploaded at PANGAEA data library: doi.org/10.1594/PANGAEA.931637.

Contribution of the candidate in % of the total workload:

Experimental concept and design:	85 %
Experimental work and acquisition of the data:	95 %
Data analysis and interpretation:	80 %
Preparation of figures and tables:	100 %
Drafting of the manuscript:	90 %

Publication II:

Title: Seasonal and inter-annual variability in the heatwave tolerance of the kelp *Saccharina latissima*

Authors: Sarina Niedzwiedz, **Nora Diehl**, Philipp Fischer, Kai Bischof

Journal: to be decided; *in preparation*

Contribution of the candidate in % of the total workload:

Experimental concept and design:	90 %
Experimental work and acquisition of the data:	5 %
Data analysis and interpretation:	75 %
Preparation of figures and tables:	20 %
Drafting of the manuscript:	60 %

Publication III:

Title: Coping with a changing Arctic: mechanisms of acclimation in the brown seaweed *Saccharina latissima* from Spitsbergen

Authors: **Nora Diehl**, Kai Bischof

Journal: Marine Ecology Progress Series, 655:43–57 (2021). doi: 10.3354/meps13532.
Raw data: uploaded at PANGAEA data library: doi.org/10.1594/PANGAEA.926970.

Contribution of the candidate in % of the total workload:

Experimental concept and design:	90 %
Experimental work and acquisition of the data:	95 %
Data analysis and interpretation:	90 %
Preparation of figures and tables:	100 %
Drafting of the manuscript:	95 %

Publication IV:

Title: Exploring intraspecific variability – Biochemical and morphological characteristics of the kelp *Saccharina latissima* along latitudinal and salinity gradients in Europe

Authors: **Nora Diehl**, Niko Steiner, Kai Bischof, Ulf Karsten, Svenja Heesch

Journal: to be decided; *in preparation*

Contribution of the candidate in % of the total workload:

Experimental concept and design:	70 %
Experimental work and acquisition of the data:	80 %
Data analysis and interpretation:	75 %
Preparation of figures and tables:	85 %
Drafting of the manuscript:	75 %

Publication V:

Title: Impacts of combined temperature and salinity stress on the endemic Arctic brown seaweed *Laminaria solidungula* J. Agardh

Authors: **Nora Diehl**, Ulf Karsten, Kai Bischof

Journal: Polar Biology, 43:647–656 (2020). doi: 10.1007/s00300-020-02668-5.
Raw data: uploaded at PANGAEA data library: doi.pangaea.de/10.1594/PANGAEA.919748.

Contribution of the candidate in % of the total workload:

Experimental concept and design:	90 %
Experimental work and acquisition of the data:	95 %
Data analysis and interpretation:	95 %
Preparation of figures and tables:	100 %
Drafting of the manuscript:	95 %

2. Publication I:

Summer heatwave impacts on the European kelp *Saccharina latissima* across its latitudinal distribution gradient

Nora Diehl, Michael Y. Roleda, Inka Bartsch, Ulf Karsten, Kai Bischof

submitted to
Frontiers in Marine Science
April 2021

Title: Summer heatwave impacts on the European kelp *Saccharina latissima* across its latitudinal distribution gradient

Authors: Nora Diehl, Michael Y. Roleda, Inka Bartsch, Ulf Karsten, Kai Bischof

Abstract

Kelps are important foundation species in coastal ecosystems experiencing severe declines worldwide associated with ocean warming. Reduced population resilience can contribute to kelp habitat loss, hence, understanding intraspecific variation in ecophysiology across its latitudinal distribution is crucial for its conservation. To investigate potential local adaptations of the broadly distributed species *Saccharina latissima* to marine heatwaves in summer, we collected sporophytes from five locations in Europe (Spitsbergen, Bodø, Bergen, Helgoland, Locmariaquer), including coldest and warmest local temperature regimes. Meristem tissue from these sporophytes was subjected to increasing temperatures of Δ+2, Δ+4 and Δ+6 °C of the respective mean summer temperatures (Δ±0 °C). Survival and corresponding physiological and biochemical traits were analyzed. Vitality (optimum quantum yield, F_v/F_m) and growth were monitored over time and biochemical responses were measured after the experiment. Growth activity was highest in northern and lowest in southern populations. Overall, samples from Spitsbergen, Bodø and Bergen were mostly unaffected by increasing summer temperatures, but the isolates from Helgoland and Locmariaquer were markedly stressed at Δ+6 °C: survival decreased, F_v/F_m was diminished, the de-epoxidation state of the xanthophyll cycle (DPS) strongly increased and the chlorophyll *a* to xanthophyll pool ratio (Chl*a*:VAZ) slightly decreased. Phlorotannins, mannitol and C:N revealed local differentiation independent of latitude. Pronounced site-specific variability of response patterns imply high phenotypic plasticity in European *S. latissima,* which are, however, not sufficient to buffer temperature stress in summer at their rear edge distribution.

Introduction

Kelps are large canopy-forming brown algae of the order Laminariales (Bartsch et al. 2008). They are important primary producers and foundation species in coastal ecosystems providing habitat, nurseries and food for many associated organisms (Dayton 1985, Bartsch et al. 2008). Strong declines of kelp forests have been reported from all over the world, including Europe (Moy & Christie 2012, Tuya et al. 2012, Voerman et al. 2013, Wernberg et al. 2015, Thomsen et al. 2019, Arafeh-Dalmau et al. 2020, Filbee-Dexter et al. 2020). Model data project a northward shift of European kelps and their disappearance at the southern distribution boundary, where drastic loss is already taking place (Müller et al. 2009, Voerman et al. 2013, Assis et al. 2018, Filbee-Dexter et al. 2020).

One widely distributed kelp along the European Atlantic is the boreal-temperate species *Saccharina latissima*, which is found from polar to temperate regions (Araújo et al. 2016). The sporophytes survive temperatures to 23 °C for a shorter period, but the mortality rate is high above 20 °C (Fortes & Lüning 1980, Bolton & Lüning 1982, Lüning 1984). The optimum growth temperature of sporophytes is between 10 and 15 °C (Bolton & Lüning 1982). *Saccharina latissima* exhibits a high degree of polymorphism and physiological plasticity, and is regarded to inherit an opportunistic growth strategy (reviewed by Bartsch et al. 2008). Nevertheless, Casado-Amezúa et al. (2019) report a decline of *S. latissima* along the northwestern Iberian Coast during the last three decades, and currently, individuals are only barely found in the North of Portugal (F. Arenas, pers. comm.). Even though the Iberian Coast represents the southernmost distribution limit of *S. latissima* in Europe, it is not the warmest location due to deep water upwelling (Lourenço et al. 2016). The highest temperatures are frequently measured in southern Brittany (Giovanni Satellite: Acker & Leptoukh 2007). Massive alterations in *S. latissima* populations have not only been recorded at their southern geographical distribution, but abundance and depth distribution has already decreased at more northern locations of Europe (Helgoland: Pehlke & Bartsch 2008, Skagerrak [southern Norway]: Bekkby & Moy 2011, southern/western Norway: Moy & Christie 2012, southern Norway and eastern USA: Filbee-Dexter et al. 2020).

Exposure to high but sublethal temperatures may result in biomass loss of *S. latissima*, e.g. due to reduced growth, which was strongly correlated with reduced carbon acquisition or due to damages in the cellular structure, such as the splitting of the medulla (Simonson et al. 2015b). High temperatures also cause changes in pigment composition and damages to the

photosynthetic apparatus (Andersen et al. 2013). Photosynthesis may, however, also be hampered by low temperatures by e.g. reducing the photosynthetic efficiency (Karsten 2007). Similarly, growth is suboptimal at low temperatures (Bolton & Lüning 1982). Consequently, we hypothesize that increasing summer temperatures will have differential effects in populations from regions with different temperature environments, while in warm regions, the lethal limits might already be surpassed or physiological responses become hampered, in colder regions, a temperature increase may support and stimulate physiological performance and growth (Davison 1987, Rautenberger & Bischof 2006, Diehl & Bischof 2021).

Saccharina latissima and other kelps use different biochemical protective and adjusting mechanisms to acclimate to temperature variations. Both high- and low-temperature stress may induce the formation of reactive oxygen species (ROS), causing intracellular strain (Collén & Davison 2001, Dring 2006, Wang et al. 2009, Zhou et al. 2010). ROS are either scavenged by antioxidants, such as the polyphenolic phlorotannins, or their formation is minimized by energy dissipation via adjustments in the xanthophyll cycle (de-epoxidation, DPS) (Amsler 2008, Wiencke & Bischof 2012 and references therein).

The effects of temperature on pigment content and composition were reported to be very complex (e.g. Machalek et al. 1996, Fernandes et al. 2016). For instance, reductions in chlorophyll *a* concentration and its ratio to chlorophyll c were found with warmer *in situ* temperatures in brown macroalgal species from Australia (Staehr & Wernberg 2009, Wernberg et al. 2016), while Andersen et al. (2013) detected contrary behavior of pigment composition in *S. latissima* with increasing temperatures (20 °C) in Europe. The total concentration of chlorophyll *a*, chlorophyll c and fucoxanthin increased significantly between 0 and 20 °C in *S. latissima* (Davison 1987). The polyol mannitol is the main photoassimilatory product in brown algae (Reed et al. 1985) and also functions as a 'compatible solute' and antioxidant (Shen et al. 1997, Jennings et al. 1998, Iwamoto & Shiraiwa 2005, Yancey 2005). C:N is a proxy for nutrient uptake (Atkinson & Smith 1983). Regardless of nutrient supply, uptake in seaweeds is affected by temperature (Roleda & Hurd 2019) and, thus, tissue C:N can deviate from seawater nutrient concentration. Decreasing C:N ratios at suboptimal temperatures (Liesner et al. 2020a, Diehl & Bischof 2021) and its correlation with seasons (Gevaert et al. 2001) has been reported for kelps in previous studies.

Overall, temperature is a major factor controlling global biogeographic patterns of marine benthic algal species (Lüning 1990, Adey & Steneck 2001, Wiencke & Bischof 2012). Hence, changes in temperature regimes, such as an increase in frequency and amplitude of marine

heatwaves (MHWs), often impair primary production or even survival, with the likely consequence of decline or even loss of important foundation communities as well as the shift into a novel ecosystem status (Frölicher et al. 2018, Harris et al. 2018, Smale et al. 2019 and references therein). Sea surface temperature (SST) has increased by 0.63 °C globally in the period 1850 – 1900 compared to 1986 – 2005 (IPCC 2019a). Under different climate change scenarios, the global mean SST is likely to increase by 1.6 °C up to more than 4 °C until the end of this century and MHWs are expected to become more severe (Frölicher et al. 2018, IPCC 2019a). MHWs are defined as periods of SST increases of 3 – 5 °C above the long-term mean, which can last from a few days to months (Meehl & Tebaldi 2004, Hobday et al. 2016). Worldwide, the annual duration of MHWs has already increased by 54 % between 1925 and 2016 (Oliver et al. 2018).

Thus, global warming frequently induces shifts in the distribution of species (King et al. 2018). Regarding temperature tolerance limits, species with a wide distribution are more tolerant than narrow-ranging species (Wiencke et al. 1994, Kelly et al. 2012, Sunday et al. 2015). In trees, species with a temperate to Arctic distribution will respond neutrally or even positively to future warming near their cold-range edge (Reich et al. 2015). This behavior might also apply to broadly distributed macroalgal species. In previous studies, different macroalgal populations are often treated as a single homogenous physiological unit, irrespective of their distribution (Reed et al. 2011). Though, local adaptation and phenotypic plasticity can result in intraspecific differences of thermal tolerance and performance to temperature, and population loss might not only occur at thermal rear edges (Bartsch et al. 2013, King et al. 2018). Recently, studies on *Laminaria digitata*, however, revealed that despite considerable plasticity at intermediate temperatures, plasticity at the upper survival limit was low (Liesner et al. 2020a,b). Thus, models assuming a uniform climatic envelope for wide-ranging species, particularly the local temperature threshold for survival, may underestimate local adaptation and extinction (Kelly et al. 2012, Filbee-Dexter et al. 2020).

The general impact of MHWs on kelps and other foundation seaweeds have been intensely studied recently (Bennett et al. 2015, Burdett et al. 2019, Nepper-Davidsen et al. 2019, Saha et al. 2020), showing increased mortality as soon as the lethal threshold of specimens is crossed with temperature anomalies (Filbee-Dexter et al. 2020). While the latter is self-evident, the understanding of thermal plasticity of species across their latitudinal distribution is still less developed (Winters et al. 2011, Jueterbock et al. 2014, Pereira et al. 2015, Wernberg et al. 2016, Liesner et al. 2020a). For instance, Wernberg et al. (2016) showed in a common

garden experiment that independent from their latitudinal distribution, the optimum photosynthetic temperature remained the same in different seaweeds in Australia, while Q_{10}-values for photosynthesis and respiration and also chlorophyll *a* concentrations decreased from cooler to warmer locations. Until now, most experimental studies on temperature stress across latitudes did not consider the different respective local temperatures. It is suggested that reduced population resilience, e.g. to potential external stressors, contributes to kelp mortality after exposure to suboptimal temperatures (Wernberg et al. 2010, Andersen et al. 2013). Bennett et al. (2019) and Filbee-Dexter et al. (2020) emphasized the relevance of understanding intraspecific variations in ecology. In this context, the underlying physiological and biochemical mechanisms are critical.

To reveal the responses of *S. latissima* to MHWs, we run a short-term experiment with field-grown sporophytes from five locations along the European Atlantic coast (Spitsbergen, Bodø, Bergen, Helgoland, Locmariaquer). We explored whether corresponding summer MHWs will have differential effects or if *S. latissima* will equally respond to increasing temperature amplitudes across all latitudes. We increased the respective local mean summer temperature (control, Δ±0 °C) in a mechanistic experimental set-up by Δ+2, Δ+4 and Δ+6 °C to investigate the relative responses to MHWs over a large geographical scale.

We hypothesized that the tolerance of European *S. latissima* to periods of summer MHWs, comprising Δ+2 – 6 °C, is related to the mean summer temperature experienced *in situ*. Since the maximum temperature during summer is lower than their physiological limit (Bolton & Lüning 1982, Lüning 1984), we expected that northern populations at their expanding edge will benefit or will not be affected (neutral response) by the temperature-amplitude treatments. Contrary, rear-edge populations at the southern limit will suffer from the temperature increase, at least after surpassing the lethal limit determined for *S. latissima* (Bolton & Lüning 1982, Lüning 1984).

Material and methods

Sampling and experimental design

Sporophytes (> 1 m) of *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders were collected in Spitsbergen (Ny-Ålesund, Norway), Bodø (North Norway), Bergen (South Norway), Helgoland (German Bight, Germany) and Locmariaquer (Brittany, France) in June 2018, 2019 and early July 2020 (**Fig. 2.1a, Table 2.1**). The map (data copyrights: Euro-Geographics for the administrative boundaries) was generated using QGIS 3.8.2-Zanzibar software (QGIS Development Team 2019).

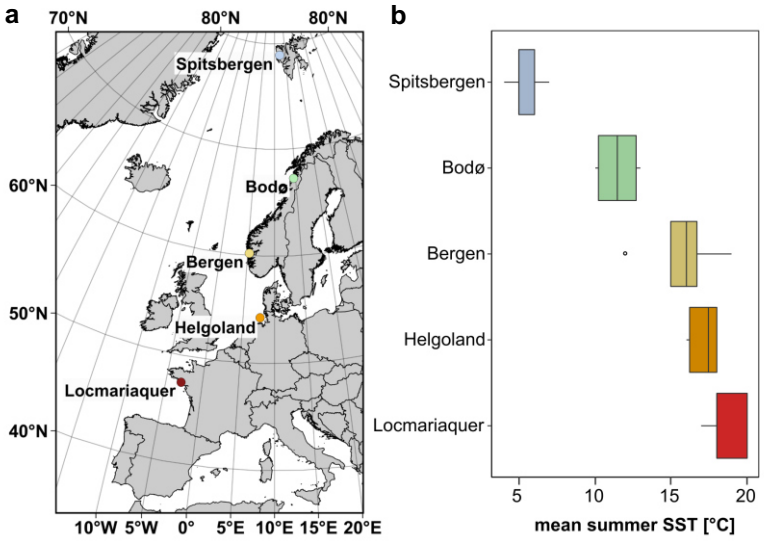


Fig. 2.1a: Sampling locations of *Saccharina latissima* (map data: ©EuroGeographics for the administrative boundaries). **b**: Mean summer (June – August) sea surface temperature (SST) from 2016 – 2020 at each sampling location based on satellite-obtained mean monthly SST data-sets (Giovanni Satellite: Acker & Leptoukh 2007).

Meristematic discs (Ø 22 – 24 mm) from 20 – 25 non-fertile sporophytes were cut at a distance of 2 – 10 cm from the stipe. Samples from Bodø, Bergen, Helgoland and Locmariaquer were transported moist, cool (< 15 °C) and dark (**Table 2.1**). All the experiments, except for the Spitsbergen material, were conducted at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. The Spitsbergen samples were processed locally at Ny-Ålesund and no transportation of the samples was necessary. For recovery and wound healing (pre-acclimation phase, **Fig. 2.2**), the samples were maintained in the control seawater temperature (**Table 2.1**). Due to logistic issues, the pre-acclimation phase varied between the sampling sites (Spitsbergen: 2 days, Bodø: 13 days, Bergen: 3 days, Helgoland: 3 days, Locmariaquer: 3 days).

Table 2.1: Summary of sampling of *Saccharina latissima*: population, North-East coordinates, *in situ* SST measured while collecting on sampling day, duration of sample transportation, duration and temperature of pre-acclimation phase, satellite-obtained mean summer SST (2016 – 2020) and respective absolute temperatures of the temperature-amplitude treatments.

population	coordinates		sampling date	*in situ* SST	transportation	pre-acclimation phase	mean summer SST[b]	temperature-amplitude treatments			
	N	E						control (Δ±0 °C)	Δ+2 °C	Δ+4 °C	Δ+6 °C
Spitsbergen	78°55.496'	011°55.108'	18.06.2019	5.7 °C	0 days	2 days at 6 °C	5.5 °C	6 °C	8 °C	10 °C	12 °C
Bodø	67°16.591'	014°34.480'	17.06.2018	9.8 °C	3 days	13 days at 10 °C	11.5 °C	12 °C	14 °C	16 °C	18 °C
Bergen	60°13.615'	005°17.207'	28.06.2018	14.6 °C	2 days	3 days at 14.5 °C	15.9 °C	16 °C	18 °C	20 °C	22 °C
Helgoland	54°10.748'	007°55.068'	26.06.2018	~15 °C[a]	1 day	3 days at 15 °C	17.5 °C	18 °C	20 °C	22 °C	24 °C
Locmariaquer	47°33.515'	002°55.468'	05.07.2020	19.6 °C	3 days	3 days at 19.5 °C	18.7 °C	19 °C	21 °C	23 °C	25 °C

[a] Data from 12 m depth, provided by the COSYNA system (Baschek et al. 2017), since they were not measured during sampling. [b] Giovanni Satellite (Acker & Leptoukh 2007).

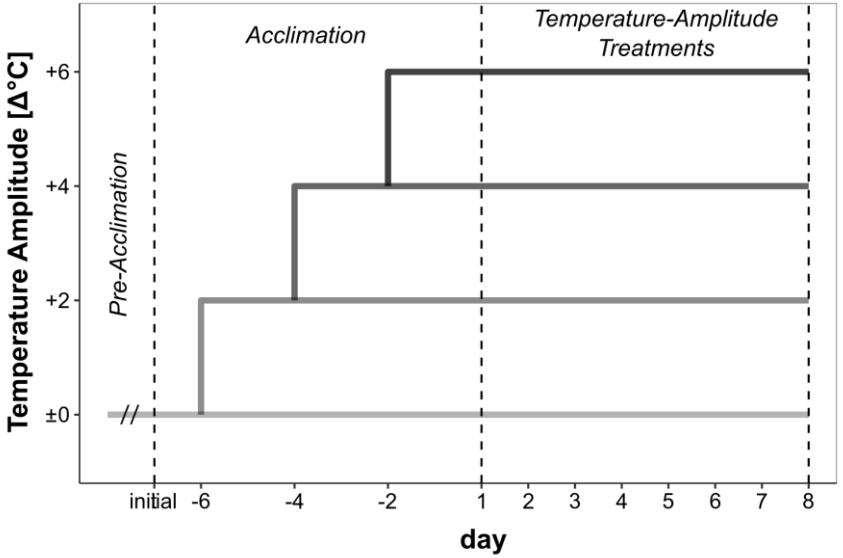


Fig. 2.2: Set-up of the temperature-amplitude experiment with *Saccharina latissima*. Pre-acclimation phase varied between the sampling sites due to logistic constraints (Spitsbergen: 2 days, Bodø: 13 days, Bergen: 3 days, Helgoland: 3 days, Locmariaquer: 3 days). During the acclimation phase, the control temperature (Δ±0 °C) was increased by 2 °C every second day to reach the temperature-amplitude treatments (Δ+2 – 6 °C). Sampling for biochemical analyses was conducted on day 8 of the temperature-amplitude treatments.

During the pre-acclimation phase, four pools of discs were prepared in separate flasks to represent the four replicates. Each pool consisted of ten meristematic discs excised from five distinct sporophytes, this means, twenty distinct sporophytes were used to ensure the independence of each replicate when subsequent sampling harvested representative discs from each pool to measure respective response variables.

In the acclimation phase (**Fig. 2.2**), the discs were successively acclimated to different temperature amplitudes (Δ+2, Δ+4, Δ+6 °C) with an increase of 2 °C every second day. We based the different absolute temperatures on the averaged mean summer sea surface temperatures (SST) at each sampling site (**Table 2.1**), using the respective mean summer SST as control (Δ±0 °C). **Figure 2.1b** displays the satellite-obtained mean summer (June – August) SST between 2016 and 2020 with a resolution of 4 × 4 km at each sampling site (Giovanni Satellite: Acker & Leptoukh 2007).

During the entire cultivation, acclimation and temperature exposure, samples were kept in aerated 2-L clear plastic bottles at 30 – 35 µmol photons m^{-2} s^{-1} (16:8 LD, ProfiLux 3 with LED Mitras daylight 150, GHL Advanced Technology, Kaiserslautern, Germany and Econlux, Solar-Stringer LED SunStrip 'daylight', Cologne, Germany) and in ½ Provasoli-enriched seawater (½ PES, Provasoli 1968, modifications: HEPES-buffer instead of Tris, double concentration of Na_2glycerophosphate, iodine enrichment after Tatewaki 1966). Water was exchanged twice a week.

All replicate discs were used to monitor survival, vitality and growth during the temperature-amplitude treatments. After the acclimation phase, first sampling was conducted (day 1, data not shown). Five discs were kept for the temperature-amplitude treatments which then run for one week (day 1 – 8, **Fig. 2.2**). For biochemical analyses samples were shock frozen in liquid N_2, stored at −80 °C, and freeze-dried (Alpha 1–4 LO plus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Statistical evaluation did not reveal any relevant significant differences in the biochemical response parameters between day 1 and day 8 (data not shown). Therefore, this study exclusively presents biochemical results after the temperature-amplitude treatments (day 8).

Physiological response variables

Growth and vitality of the samples was monitored as physiological response variables and the survival of the discs was observed.

All discs were counted at the end of the experiment for calculating the survival of the discs between day 1 and day 8 in % (**Fig. 2.2**). The algal discs were categorized as 'dead' when they started to decompose (**supplement Fig. S2.1**).

For growth analysis, the discs were photographed and their size analyzed with ImageJ (Version 1.52a, Java 1.8.0_112, Wayne Rasband, National Institute of Health, USA), using a 3 × 3 cm square as a size reference. As the samples grew with different rates prior to the temperature-amplitude treatments, the initial size of the replicates was adjusted to 100 % and the increase in size calculated as % of initial for a better comparison of algal growth.

As proxy for photosynthetic performance and, hence, of algal vitality, the *in vivo* chlorophyll fluorescence of photosystem II (optimum quantum yield, F_v/F_m) was measured after 5 min of dark acclimation using an pulse-amplitude-modulated fluorometer (Imaging-PAM, Walz GmbH Mess- und Regeltechnik, Effeltrich, Germany), which was set up to determine the amplitude of the initial fluorescence signal (F_t) between 0.15 and 0.2 (SP intensity = 8, SP duration = 3 s), as recommended in the manual (Imaging-PAM M-Series Chlorophyll Fluorometer, Heinz Walz GmbH, Effeltrich, Germany). F_v/F_m was monitored in all stages of visible stress, even until the tissue was completely decomposed.

Biochemical response variables

Pigments

Photosynthetic and accessory pigments were extracted and analyzed following Koch et al. (2015), applying little modifications as follows: 50 – 150 mg of freeze-dried and ground samples were extracted in 1 mL 90 % aqueous acetone (v/v) in darkness at 4 °C for 24 h. Afterwards, the samples were centrifuged (13,000 g; 5 min, 4 °C), the supernatant filtered. Pigments were separated by a Spherisorb® ODS-2 column (250 × 4.6 mm, 5 µm, Waters, Milford, MA, USA) with a HPLC (LaChromElite® system, L-2200 autosampler [chilled], DA-detector L-2450, VWR-Hitachi International GmbH, Darmstadt, Germany), applying a gradient according to Wright et al. (1991). Standards of chlorophyll *a* (Chl *a*), chlorophyll *c*2, fucoxanthin, violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) (DHI Lab Products, Hørsholm, Denmark) were used to identify and quantify the peaks at λ 440 nm (software: EZChrom Elite,

Agilent Technologies, Santa Clara, CA, USA, Version 3.1.3., 2004). Chl *a* and the accessory pigment pool (Acc) are expressed in mg g^{-1} dry weight (DW), the pool size of the xanthophylls (VAZ) in µg g^{-1} DW. To determine differentiations in the photosynthetic apparatus, the ratios Chl*a*:Acc and Chl*a*:VAZ were calculated. The de-epoxidation state of the xanthophyll cycle (DPS) was calculated after Colombo-Pallotta et al. (2006):

$$DPS = \frac{Z + 0.5A}{V + A + Z}$$

Phlorotannins

The total phlorotannin content was determined using the Folin-Ciocalteu method described by Cruces et al. (2012), following Springer et al. (2017). For this, 10 – 15 mg of freeze-dried and ground samples were extracted in 1 mL of 70 % aqueous acetone (v/v) for 24 h in darkness at 4 °C and with constant shaking. After centrifugation (2,500 g; 10 min), 250 µL dH_2O, 200 µL 20 % sodium carbonate (Na_2CO_3) and 100 µL 2N Folin-Ciocalteu reagent (Sigma-Aldrich, Seelze, Germany) were added to 50 µL of each supernatant. After 45 min of incubation in darkness, the absorbance at λ 730 nm was read in a microplate reader (FLUOstar OPTIMA, BMG Labtech). Purified phloroglucinol ($C_6H_6O_3$, Sigma-Aldrich, Seelze, Germany) was used for calibration and the total soluble phlorotannin concentrations of the samples were normalized to mg g^{-1} DW.

Mannitol

The mannitol concentration was determined after Diehl et al. (2020), using the method described in Karsten et al. (1991). Approx. 15 mg of freeze-dried and ground sample were extracted in 1 mL 70 % aqueous ethanol (v/v) and incubated in a water bath at 70 °C for 3 – 4 h. After centrifugation (13,000 g; 5 min), 800 µL of the supernatant were evaporated to dryness (Alpha 1–4 LSCplus and RVC 2–25 CDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and the pellets re-dissolved in 800 µL HPLC grade water. The samples were then centrifuged for another 5 min (13,000 g) and the obtained supernatant analyzed in an HPLC (Agilent Technologies system 1200 Series, RI-Detector (35 °C), Santa Clara, California, USA) with an Aminex Fast Carbohydrate Analysis Column HPAP (100 × 7.8 mm, 9 µm, BioRad, Munich, Germany), protected by a guard cartridge (Phenomenex, Carbo-Pb-2+ 4 × 3.00 mm i.d., Aschaffenburg, Germany). The flow rate of the mobile phase (100 % dH_2O) was adjusted to 1 mL min^{-1} at 10 – 100 bar and 70 °C. Purified D-mannitol ($C_6H_{14}O_6$, Roth) was used as standard. Mannitol contents were expressed in mg g^{-1} DW.

C:N ratio, carbon and nitrogen

Total carbon (C), total nitrogen (N) content and the C:N ratio were analyzed following the protocol of Graiff et al. (2015). 2 – 3 mg of lyophilized and ground samples were weighed into tin cartridges (6 × 6 × 12 mm) and combusted at 950 °C. An elemental analyzer (Vario EL III, Elementar, Langenselbold, Germany) automatically quantified the absolute content of C and N, using acetanilide (C_8H_9NO) as standard (Verardo et al. 1990). Total C and total N content were expressed in mg g^{-1} DW.

Statistics

All data-sets were tested for normal distribution (Shapiro-Wilk test, $p > 0.05$) and homogeneity of variance (Levene's test, $p > 0.05$) and transformed if required. Outliers (R-Studio Bonferroni, $p < 0.05$) were excluded from further analyzes. The increase of size over time was analyzed with repeated measures two-way ANOVA (population × temperature-amplitude) followed by a post hoc Tukey's test. Since the F_v/F_m data-set was non-parametric, it was analyzed using Friedman test followed by a post hoc Wilcoxon signed-rank test. The impact of sampling locations (populations) and temperature-amplitudes on survival and the biochemical response variables at the end of the experiment were tested with two-way ANOVAs followed by post hoc Tukey's tests. For non-parametric data, the data-sets were split, and afterwards, the impact of different populations or temperature-amplitudes were tested individually by one-way ANOVA with a post hoc Tukey's test (parametric) or Kruskal-Wallis tests with the Dunn-Bonferroni's post hoc test (non-parametric). The level of significance was set to $p < 0.05$. Significances are marked by different letters; lowercase letters mark within-subjects effects and uppercase letters between-subjects effects. All statistical analyzes were conducted with RStudio (Version 1.3.1073, 2020, Boston, MA, USA). Details from statistical analyzes are presented in the electronic supplement (**Table S2.2 – 2.7**). Pearson correlations between dependent parameters, as well as between all parameters and the absolute temperatures, were calculated and interpreted after Cohen (1988). The statistical results are summarized in **Table 2.2**.

Table 2.2: Pearson correlations between dependent parameters of *Saccharina latissima* and between each parameter and absolute temperatures at the end of the temperature-amplitude experiment. $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***.

correlated factors	*r*	*df*	*t*	*p*
abs. temp – survival	-0.403	78	-3.890	< 0.001***
abs. temp – increase size (day 8)	-0.796	78	-11.595	< 0.001***
abs. temp – F_v/F_m (day 8)	-0.445	78	-4.395	< 0.001***
abs. temp – Chl *a*	-0.058	78	-0.512	0.610
abs. temp – Acc	-0.124	78	-1.103	0.274
abs. temp – VAZ	0.175	78	1.571	0.120
abs. temp – DPS	0.435	78	4.267	< 0.001***
abs. temp – Chl*a*:Acc	0.090	78	0.796	0.428
abs. temp – Chl*a*:VAZ	-0.177	78	-1.591	0.116
abs. temp – phlorotannins	0.605	78	6.712	< 0.001***
abs. temp – mannitol	-0.145	78	-1.295	0.199
abs. temp – C:N	0.279	77	1.522	0.013*
abs. temp – C	0.347	76	2.352	0.002**
abs. temp – N	0.017	76	0.092	0.885
F_v/F_m – survival (both day 8)	0.669	78	7.945	< 0.001***
F_v/F_m – increase size (both day 8)	0.233	78	2.112	0.038*
C:N – C	0.270	76	2.441	0.017*
C:N – N	-0.729	76	-9.273	< 0.001***
C – phlorotannins	0.365	76	3.421	0.001**
C – mannitol	0.040	76	0.348	0.729

Results

Survival among different populations from the species northern to southern distribution range did not significantly vary in response to increasing temperature amplitudes (**Table 2.3**, Kruskal-Wallis test: $p = 0.022$, reject H_0 if $p \leq$ alpha/2, alpha = 0.05), but decreased survival at higher absolute temperatures was determined ($r = -0.40$, $p < 0.001$). Samples from Spitsbergen showed survival of 100 % (absolute temperatures 6 – 12 °C) and samples from Bodø (12 – 18 °C) and Bergen (16 – 22 °C) of 95 – 100 %, while samples collected at the locations with highest mean summer temperatures – Helgoland and Locmariaquer – died and decomposed at the Δ+6 °C (Helgoland: 24 °C, 70 % survival; Locmariaquer: 25 °C, 65 % survival) (Kruskal-Wallis test: $p_{Helgoland} = 0.078$, $p_{Locmariaquer} = 0.016$, reject H_0 if $p \leq$ alpha/2).

Table 2.3: Survival (%), chlorophyll *a* (Chl *a*; mg g^{-1} dry weight [DW]), pool size of accessory pigments (Acc; mg g^{-1} DW) and pool size of xanthophylls (VAZ; µg g^{-1} DW), mannitol (mg g^{-1} DW) and C:N ratio of *Saccharina latissima* across latitudes in Europe after the temperature-amplitude experiment. Experimental set-up: temperature-amplitude treatment (Δ°C) and respective absolute temperature (°C). Values are means ± SD (n = 4). Significant differences between the temperature treatments within each population are marked by different lowercase letters and between the populations by different uppercase letters (parametric data: two-way ANOVA with post hoc Tukey's test; non-parametric data: one-way ANOVA with post hoc Tukey's test or Kruskal-Wallis test with post hoc Dunn-Bonferroni's test; $p < 0.05$).

population	treatment (Δ°C)	abs. temp. (°C)	survival (%)		Chl *a* (mg g^{-1} DW)		Acc (mg g^{-1} DW)		VAZ (µg g^{-1} DW)		C:N		mannitol (mg g^{-1} DW)	
Spitsbergen	±0	6	100 a	A	0.69 ± 0.12 a	A	0.57 ± 0.07 a	A	66.6 ± 14.2 a	A	232.8 ± 65.5 a	AB	17.6 ± 3.8 a	A
	+2	8	100 a		0.81 ± 0.20 a		0.67 ± 0.14 a		88.0 ± 23.3 a		145.5 ± 51.6 a		14.9 ± 1.0 a	
	+4	10	100 a		0.73 ± 0.41 a		0.59 ± 0.31 a		69.6 ± 41.0 a		80.7 ± 54.2 a		15.0 ± 1.4 a	
	+6	12	100 a		0.93 ± 0.28 a		0.71 ± 0.11 a		71.2 ± 7.6 a		155.8 ± 57.6 a		16.0 ± 1.4 a	
Bodø	±0	12	100 a	A	1.53 ± 0.25 a	B	1.40 ± 0.18 a	B	49.5 ± 6.5 a	B	251.5 ± 34.4 a	AC	18.0 ± 1.0 a	AB
	+2	14	100 a		1.57 ± 0.25 a		1.37 ± 0.17 a		58.2 ± 3.9 a		235.9 ± 35.7 a		17.5 ± 1.7 a	
	+4	16	95 a		1.28 ± 0.36 a		1.17 ± 0.15 a		42.5 ± 12.5 a		209.3 ± 30.0 a		17.6 ± 1.1 a	
	+6	18	100 a		1.51 ± 0.15 a		1.29 ± 0.19 a		55.1 ± 6.0 a		156.1 ± 39.7 a		16.7 ± 0.9 a	
Bergen	±0	16	100 a	A	1.11 ± 0.23 a	C	0.91 ± 0.12 a	C	82.6 ± 19.9 a	A	173.5 ± 50.0 a	BD	14.3 ± 1.5 a	A
	+2	18	95 a		1.20 ± 0.33 a		0.94 ± 0.17 a		84.5 ± 21.7 a		202.1 ± 39.4 a		13.0 ± 1.7 a	
	+4	20	100 a		1.04 ± 0.13 a		0.81 ± 0.06 a		82.7 ± 17.3 a		109.9 ± 25.6 a		15.5 ± 1.6 a	
	+6	22	100 a		1.22 ± 0.27 a		0.92 ± 0.16 a		98.7 ± 21.7 a		90.6 ± 37.1 a		14.2 ± 5.0 a	
Helgoland	±0	18	100 a	A	1.25 ± 0.21 a	C	0.92 ± 0.16 a	C	67.6 ± 16.1 a	A	210.4 ± 49.8 a	C	22.6 ± 3.5 a	B
	+2	20	95 a		1.21 ± 0.18 a		0.85 ± 0.11 a		63.5 ± 7.5 a		261.2 ± 71.3 a		22.0 ± 3.2 a	
	+4	22	95 a		1.25 ± 0.08 a		0.84 ± 0.07 a		75.8 ± 5.1 a		158.4 ± 80.6 a		21.1 ± 4.1 a	
	+6	24	70 a		0.96 ± 0.28 a		0.63 ± 0.14 a		108.3 ± 27.5 a		210.1 ± 74.0 a		22.8 ± 3.0 a	
Locmariaquer	±0	19	100 a	A	0.71 ± 0.19 a	A	0.55 ± 0.14 a	A	54.8 ± 15.7 a	AB	95.2 ± 28.1 a	D	16.0 ± 2.4 a	A
	+2	21	100 a		0.58 ± 0.38 a		0.46 ± 0.22 a		50.7 ± 25.0 a		158.2 ± 33.7 a		18.3 ± 1.1 a	
	+4	23	100 a		0.72 ± 0.26 a		0.56 ± 0.18 a		61.0 ± 12.6 a		162.5 ± 43.5 a		18.7 ± 1.6 a	
	+6	25	65 a		0.42 ± 0.27 a		0.60 ± 0.20 a		89.7 ± 29.8 a		27.6 ± 53.7 a		15.3 ± 2.9 a	

For all five populations, size of the discs (**Fig. 2.3**) increased over time ($p < 0.001$). There were significant differences in growth between the populations (P) ($p < 0.001$) and a strong negative correlation with absolute temperature (r = −0.80, $p < 0.001$). While Spitsbergen samples grew considerably reaching 160 – 200 % of their initial size, samples from all other populations exhibited considerably reduced growth activity (< 130 %), being lowest in Helgoland and Locmariaquer. Differences between samples from Bodø and Bergen were low and non-significant. Even though statistical significances were also detected for the temperature-amplitude treatments (TA) ($p < 0.001$) and P×TA interaction ($p < 0.001$), these could not be assigned to temperature increase per se, as for instance, growth in Spitsbergen Δ+2 °C was significantly higher than Δ+6 °C, whereas Δ±0 and Δ+6 °C did not differ significantly.

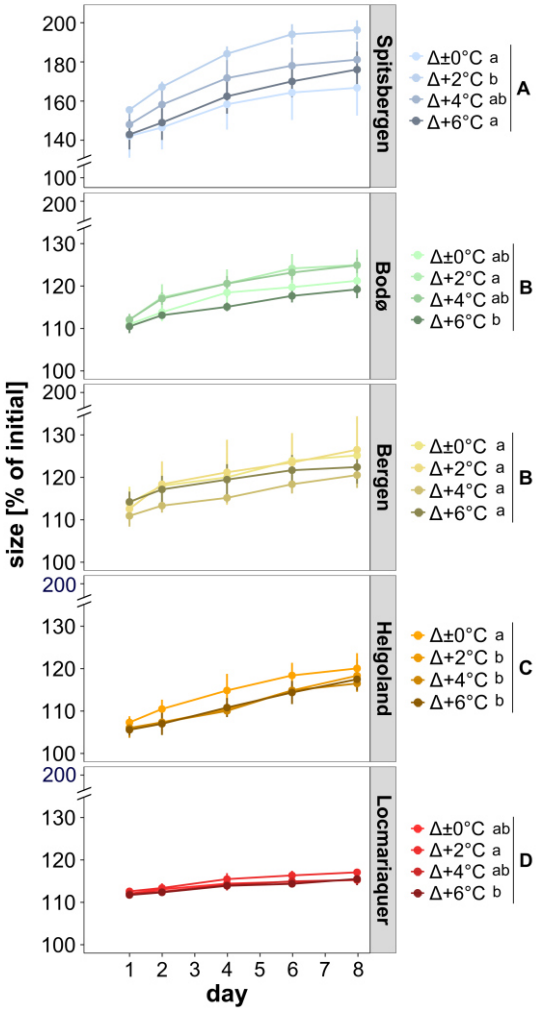


Fig. 2.3: Size (% of initial) of meristematic discs of *Saccharina latissima* across latitudes in Europe during the temperature-amplitude experiment. Experimental set-up: mean summer SST (control, Δ±0 °C) and temperature stress treatments: Δ+2, Δ+4, Δ+6 °C. Values are means ± SD ($n = 4$). Significant differences are marked by different letters; lowercase letters mark within-subjects effects and uppercase letters between-subjects effects (two-way ANOVA with post hoc Tukey's test; $p < 0.05$).

We measured significant differences in optimum quantum yield (F_v/F_m) over time between the five populations ($p < 0.001$) (**Fig. 2.4**). The data-set could not be tested for P×TA interaction. Survival and growth (increase in size) responses are supported by F_v/F_m measurements on day 8 ($r_{survival} = 0.67$, $p_{survival} < 0.001$, $r_{growth} = 0.23$, $p_{growth} < 0.05$). However, all samples from Spitsbergen, Bodø and Bergen (6 – 22 °C), and the Δ±0, Δ+2 and Δ+4 °C treatments from Helgoland (18 – 22 °C) and Locmariaquer (19 – 23 °C) revealed F_v/F_m values > 0.6 throughout the experiment, whereas significant decreases in optimum quantum yield were detected already after the temperature acclimation phase (day 1) in samples from Helgoland and Locmariaquer at Δ+6 °C (> 24 °C) ($p < 0.05$). A significant impact of TA was determined in all populations ($p < 0.001$). Higher F_v/F_m values were detected in the samples from Spitsbergen with increasing temperatures of Δ+6 °C ($p < 0.05$). Contrary, *S. latissima* from Bergen, Helgoland and Locmariaquer were negatively affected by all TA as those samples revealed lower F_v/F_m values with increasing temperatures between Δ±0 and Δ+6 °C ($p < 0.05$). The optimum quantum yield on day 8 decreased with increasing absolute temperatures ($r = -0.45$, $p < 0.001$), leading to an intensified negative effect of TA on F_v/F_m values with decreasing latitudes: Quantum yield in samples from Bergen only diminished at Δ+6 (22 °C) and not at Δ+4 (20 °C) and Δ+2 °C (18 °C), while samples from Helgoland already significantly decreased at Δ+2/Δ+4 °C (20/22 °C, non-significant to each other) with a strong significant decline at Δ+6 °C (24 °C). A conspicuous pattern of decreasing F_v/F_m values, with significant differences between all treatments from Δ±0 °C to Δ+6 °C (19 – 25 °C) was determined in samples from Locmariaquer.

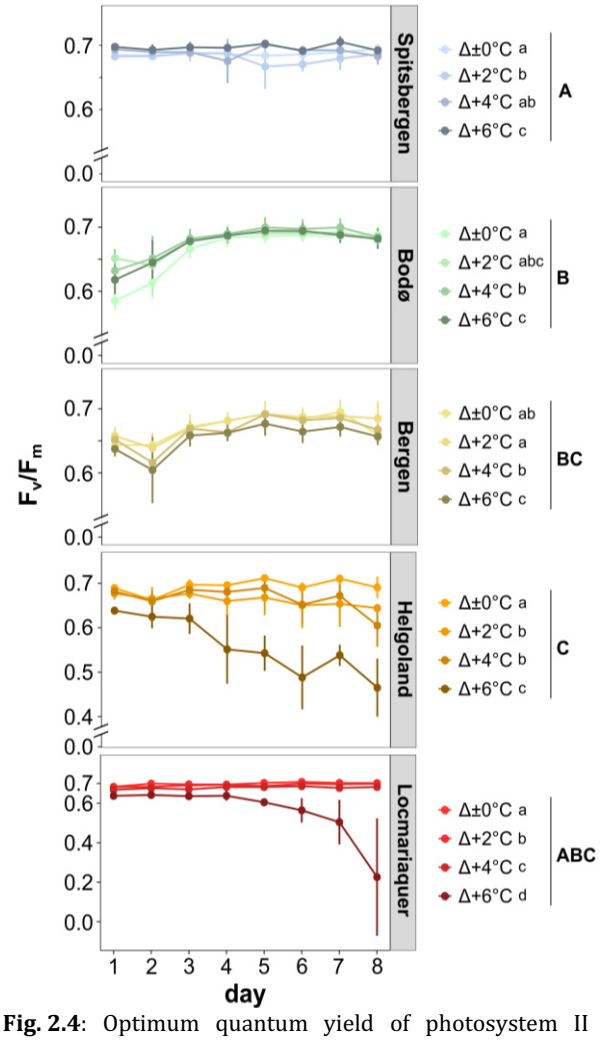


Fig. 2.4: Optimum quantum yield of photosystem II (F_v/F_m) of *Saccharina latissima* across latitudes in Europe during the temperature-amplitude experiment. Experimental set-up: mean summer SST (control, Δ±0 °C) and temperature stress treatments: Δ+2, Δ+4, Δ+6 °C. Values are means ± SD ($n = 4$). Significant differences are marked by different letters; lowercase letters mark within-subjects effects and uppercase letters between-subjects effects (Friedman test with post hoc Wilcoxon signed rank test; $p < 0.05$).

The absolute pigment contents (Chl *a*, Acc) sampled on day 8 did not reveal any trends assigning to absolute temperatures or the latitudinal distribution of *S. latissima* (**Table 2.3**). There also was no significant impact of TA and no P×TA interaction. The TA, in general, had no significant effect on VAZ, DPS, Chl*a*:Acc or Chl*a*:VAZ (**Fig. 2.5, Table 2.3**). Data-sets could not be tested for interaction. Still, a trend to higher VAZ concentrations in the Δ+6 °C treatments from Helgoland and Locmariaquer (> 24 °C) was observed (**Table 2.3**).

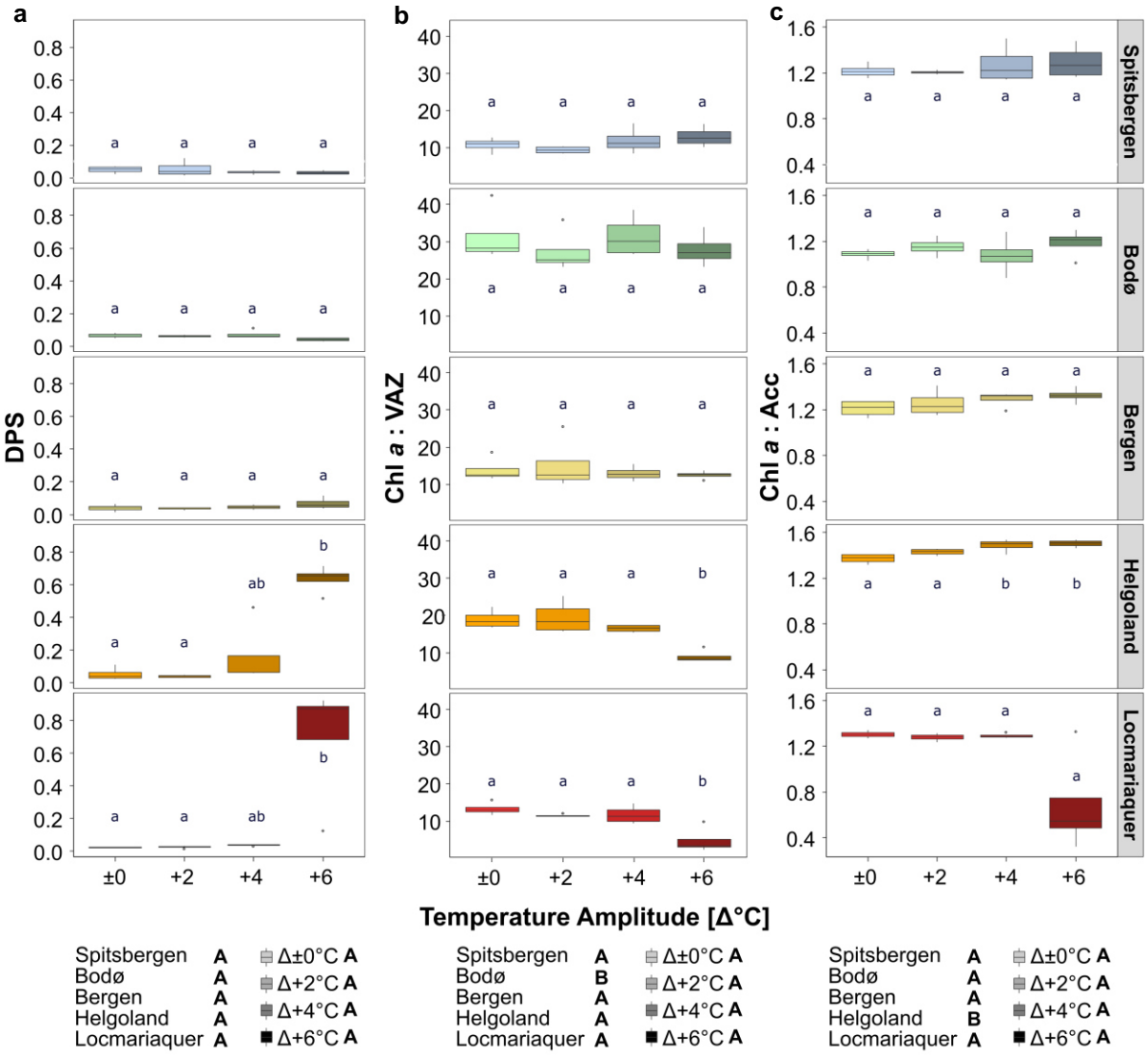


Fig. 2.5a: De-epoxidation state of the xanthophyll cycle (DPS), **b:** chlorophyll *a* : pool size of xanthophylls (Chl*a*:VAZ) and **c:** chlorophyll *a* : accessory pigments ratio (Chl*a*:Acc) of *Saccharina latissima* across latitudes in Europe after the temperature-amplitude experiment. Experimental set-up: mean summer SST (control, Δ±0 °C) and temperature stress treatments: Δ+2, Δ+4, Δ+6 °C (n = 4). The bottom line of the box represents the 25th percentile, the center line of the box the median and the top line of the box the 75th percentile. The lower and upper whiskers represent the lowest and the highest values in the data. Significant differences are marked by different letters; lowercase letters mark within-subjects effects and uppercase letters between-subjects effects (one-way ANOVA with post hoc Tukey's test or Kruskal-Wallis test with post hoc Dunn-Bonferroni's test; $p < 0.05$).

The de-epoxidation state of the xanthophyll cycle (DPS, **Fig. 2.5a**) of the samples from Helgoland and Locmariaquer both increased significantly at Δ+6 °C ($p < 0.05$) and a positive correlation between DPS and absolute temperatures was detected ($r = 0.44$, $p < 0.001$). Even though absolute Chl *a* and VAZ content were both not affected during the experiment (**Table 2.3**), the ratio of Chl*a*:VAZ (**Fig. 2.5b**) significantly decreased at Δ+6 °C in the samples from Helgoland and Locmariaquer ($p < 0.001$) and was significantly higher in all samples from Bodø ($p < 0.001$). Further major changes in the photosynthetic pigment settings under the TA or a correlation with absolute temperatures were not apparent, even so in the Chl*a*:Acc ratio (**Fig. 2.5c**). Though, Chl*a*:Acc of the Helgoland samples was significantly higher after the experiment than of samples from the other populations ($p < 0.001$).

None of the other biochemical response variables – mannitol, C:N ratio, phlorotannins – were significantly affected by the TA (**Fig. 2.6**, **Table 2.3**), but significant differences were present between samples from the five locations in all three parameters ($p < 0.001$), however, without meaningful correlation to the sampling latitude. Regarding phlorotannins (**Fig. 2.6**), the lowest concentrations were found in the samples from Spitsbergen and highest in the samples from Bergen and Locmariaquer. Generally, the phlorotannin content was higher at higher absolute temperatures ($r = 0.61$, $p < 0.001$). Overall, mannitol concentrations (**Table 2.3**) were similar in all populations, though the samples from Locmariaquer exhibited the lowest concentrations, no correlation to absolute temperatures was determined. *Saccharina latissima* from Helgoland exceeded C:N ratios above 20, while samples from all other populations had values of about 13 – 19, independently of the TA (**Table 2.2**). These differences were mainly based on significantly lower nitrogen (N) concentrations in the Helgoland samples and not on variations in total carbon (C) (**Supplement Tables S2.1 and S2.5**). Total C had only little impact on C:N ($r = -0.27$, $p < 0.05$), while total N revealed strong correlation ($r = -0.73$, $p < 0.001$). C:N ratio and total C, however, both increased with higher temperatures ($r_{C:N} = 0.28$, $p_{C:N} < 0.05$, $r_C = 0.35$, $p_C < 0.01$). Positive correlation was detected between total C and phlorotannins ($r = 0.37$, $p < 0.01$), but not mannitol.

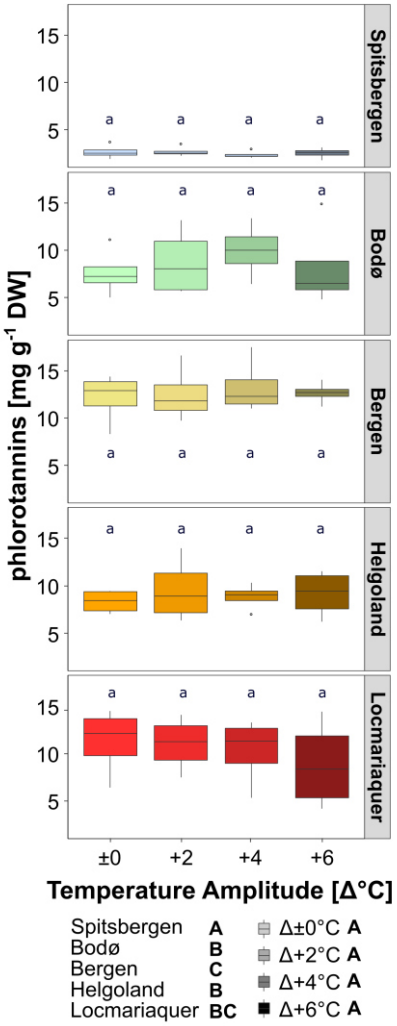


Fig. 2.6: Phlorotannin concentration (mg g^{-1} dry weight [DW]) of *Saccharina latissima* across latitudes in Europe after the temperature-amplitude experiment. Experimental set-up: mean summer SST (control, Δ±0 °C) and temperature stress treatments: Δ+2, Δ+4, Δ+6 °C (n = 4). The bottom line of the box represents the 25th percentile, the center line of the box the median and the top line of the box the 75th percentile. The lower and upper whiskers represent the lowest and the highest values in the data. Significant differences are marked by different letters; lowercase letters mark within-subjects effects and uppercase letters between-subjects effects (two-way ANOVA with post hoc Tukey's test; $p < 0.05$).

Discussion

Impacts of short-term temperature increase on Saccharina latissima *across latitudes*

This is the first study revealing the performance of *Saccharina latissima* along its north-south distribution gradient to relative marine heatwaves (MHWs) in summer. We simulated heat stress by applying temperature amplitudes of Δ+2 – 6 °C on top of local mean summer sea surface temperature (SST) (Δ±0 °C). It became evident that *S. latissima* reveals intraspecific differences in physiological and biochemical levels to temperature increase across latitudes. However, we did not detect homogenous effects of the temperature amplitudes, meaning none of the parameters tested responded in the same way to an increase of Δ+2, Δ+4 or Δ+6 °C in the different populations. We detected harmful impacts of increasing temperatures on physiology and biochemical status exclusively in the southernmost samples of *S. latissima*, for which the temperature-amplitude treatments exceeded 20 °C. Northern isolates overall were not negatively affected. Nevertheless, we also found strong site-specific differences, which were especially prominent in growth and phlorotannin content.

Survival of *S. latissima* across latitudes was not significantly affected by a temperature increase of Δ+2 – 6 °C. Increased mortality of *S. latissima* sporophytes was already reported for temperatures above 20 °C (Fortes & Lüning 1980, Bolton & Lüning 1982, Lüning 1984) and blade tissue of *S. latissima* from Nova Scotia was severely damaged and decomposed after one-week exposure to high temperatures (18 and 21 °C) (Simonson et al. 2015b). Contrary to these studies, samples from Helgoland and Locmariaquer in our experiment survived a temperature increase by Δ+2 to Δ+4 °C (absolute temperatures up to 23 °C) and revealed high mortality only in the Δ+6 °C treatment (≥ 24 °C) after 8 days of treatment. Thus, *S. latissima* from these two locations showed extraordinary resilience to MHWs in summer, although the chosen methodology of temperature increase might have influenced the results: The stepwise increase of temperature as conducted in our experimental set-up, which should simulate the situation in nature, allowing for a stepwise acclimation and, hence probably helped to survive unfavorable conditions (Terblanche et al. 2007). Thus, the published upper survival temperatures derived from experiments without pre-acclimation phase (Fortes & Lüning 1980, Bolton & Lüning 1982, Lüning 1984, Simonson et al. 2015a) and constant temperatures may not reveal the actual resilience present in nature where kelps are normally subjected to slowly increasing temperature amplitudes. Hence, we can conclude that *S. latissima* at their warm-edge distribution can withstand short periods of summer MHWs up to 25 °C, although mortality extensively increases at > 23 °C. We monitored survival only using meristematic discs,

although kelp thalli under heat stress usually start degenerating from the tip (Franke 2019, I. Bartsch pers. comm.). Furthermore, adult meristematic field tissue might be more resilient than laboratory cultures or young meristems (Hanelt et al. 1997, Heinrich et al. 2016).

Along the entire latitudinal range, the growth of the samples was not affected by temperature amplitudes but differed between the populations. In contrast to previous studies working within comparable temperature ranges, we did not detect enhanced growth with warmer temperatures in the Arctic samples (Olischläger et al. 2017 [4 and 10 °C], Li et al. 2020 [0, 8 and 15 °C], Diehl & Bischof 2021 [4, 6, 8 and 10 °C]), since Δ±0 °C (absolute temperature 6 °C) and Δ+6 °C (12 °C) revealed no significant differences. However, Olischläger et al. (2017) and Li et al. (2020) worked with juvenile laboratory-grown sporophytes, which are most likely more sensitive to environmental variations (Hanelt et al. 1997, Heinrich et al. 2016). Strong site-specific differences in growth became evident in our study with overall diminished growth at higher temperatures. Growth activity in samples from Spitsbergen was highest by far, even though the control temperature (Δ±0 °C, absolute temperature 6 °C) and the Δ+2 °C treatment (8 °C) were below the temperature range of 10 – 15 °C which was reported as optimum growth temperature for *S. latissima* (Bolton & Lüning 1982). Growth was significantly lower in samples from Helgoland (18 – 24 °C), which were exposed to temperatures highly exceeding the optimum growth temperature of the species, than from Bodø (12 – 18 °C) and Bergen (16 – 22 °C). Isolates from Locmariaquer (19 – 25 °C), which were exposed to slightly higher temperatures than the Helgoland population, exhibited the lowest growth activity and thereby are in accordance with expectations from published evidence (Bolton & Lüning 1982). Growth of *S. latissima* generally is initiated in late winter after the termination of the fertile season resulting in rapid growth in early spring (Bartsch et al. 2008). This has been followed specifically from Helgoland and Norway (Helgoland: Lüning 1979, Norway [mainland]: Sjøtun 1993, Norway [Spitsbergen]: I. Bartsch pers. comm.). Over summer, growth of *S. latissima* is substantially reduced, as was observed at Helgoland, though it does not completely cease (Lüning 1979). Growth of *S. latissima* is closely dependent on light availability (Dunton 1985), resulting in higher growth rates during longer days (Fortes & Lüning 1980) and is sensitive to the onset of short day length, which lead to an immediate reduction in growth and initiation of fertility (Lüning 1988). As we collected all samples in summer in day lengths > 15 hours, all meristems should have been in a general mode of some growth activity. Spurkland & Iken (2011, 2012) suggest that *S. latissima* exhibits growth plasticity within a genetically fixed seasonal growth window that might be an advantage in locations with high environmental variability, such as the Arctic. The particular irradiance conditions

in the Arctic may have promoted the extraordinary growth activity of the Spitsbergen population. Consequently, observed growth activity might have been more dependent on internal seasonal growth patterns than on their exposure temperatures during the experiment, resulting in *S. latissima* from the Arctic growing most and from Locmariaquer least in summer.

Temperature increase affected the optimum quantum yield of *S. latissima*, even though the effects were not consistent with the amplitudes but with the absolute temperatures. While samples from Spitsbergen were even stimulated at relatively higher summer temperatures of Δ+6 °C (absolute temperature of 12 °C), the samples from warmer locations clearly suffered from Δ+6 °C treatments (> 20 °C), and this negative effect of temperature increase intensified with decreasing latitudes. Several studies already detected an increase in optimum quantum yield of Arctic *S. latissima* with increasing temperatures up to 10 °C (Iñiguez et al. 2016, Diehl & Bischof 2021), but a decrease at temperatures above 20 °C as investigated in southern Norwegian and Danish populations (Andersen et al. 2013, Nepper-Davidsen et al. 2019). In contrast, independent from their origin, our samples exhibited F_v/F_m values of > 0.6 at temperatures up to 20 °C and even higher, which indicates a good physiological status (Dring et al. 1996) and thus a good capacity to withstand short-term SST increases even during summer at all locations. It is striking that *S. latissima* from Locmariaquer revealed better photosynthetic quantum yields than the Helgoland population, although both were kept at comparable high temperatures. Locmariaquer samples remained good physiological status at 23 °C (> 0.6), while F_v/F_m values already declined at 22 °C in samples from Helgoland, similar to the observed survival in our study.

The tested temperature amplitudes had no effect on pigment concentrations and composition, except for Helgoland and Locmariaquer, for which Δ+6 °C (> 24 °C) surpassed sublethal thresholds imposing considerable stress (Bolton & Lüning 1982, Lüning 1984). Even though chlorophyll *a* (Chl *a*) and the pool size of the xanthophyll cycle (VAZ) did not significantly change with amplitudes, the Chl*a*:VAZ ratio decreased at Δ+6 °C in samples from Helgoland and Locmariaquer due to slightly lower Chl *a* and higher VAZ concentrations. Chl *a* is reported to decrease at suboptimal temperatures resulting in changes of the photosynthetic apparatus and consequential reduced photosynthetic activity under warmer temperatures (Wernberg et al. 2016, Nepper-Davidsen et al. 2019). By interconversion of violaxanthin to zeaxanthin (de-epoxidation state, DPS), the xanthophyll cycle acts as precursory protective mechanisms for dissipation of excessive energy, thereby preventing the generation of reactive oxygen species (ROS) (Goss & Jakob 2010). Comparable to Nepper-Davidsen et al. (2019) we could detect a strong increase of DPS at high temperatures in samples from Helgoland and Locmariaquer,

indicating physiological exertion in *S. latissima*. The increase of VAZ in the same treatments is reflecting the importance of DPS as a protective mechanism at high temperatures. We did not detect a clear link of latitudinal distribution or absolute temperatures to variation in pigments. Though, except for Spitsbergen, a trend for decreasing Chl *a* and accessory pigment (Acc) concentration in samples from locations with higher mean summer SST was determined, but the ratio between Chl*a*:Acc remained almost the same in all samples. As pigment composition is largely depending on the light environment, potential differences existent *in situ* might have been diminished by the experimental design applying the same photoperiod and irradiance conditions to all samples over > 14 days. The pre-acclimation phase was rather long (partially), except for Spitsbergen, which could explain the overall lower Chl *a* and Acc concentrations in samples from this population.

Nutrient uptake in seaweeds is affected by temperature (Roleda & Hurd 2019). In our experiment, C:N ratio did not vary in the temperature-amplitude treatments and no pattern of intracellular nitrogen (N) was detected across latitudes since generally, local variations were very small. In our study, variations in C:N were mainly based on N and only a little on total carbon (C). N is frequently limited in coastal systems over summer. However, the samples from Helgoland exclusively revealed C:N ratios of above 20, indicating N limitation despite high supply with N during the experiment (Atkinson & Smith 1983). Enhanced N supply supports heat tolerance in kelp species (Gerard 1997b, Fernández et al. 2020), thus, the exposure to ½ PES as in our study might have ameliorated negative impacts of enhanced temperatures. C:N ratios and C content increased at higher absolute temperatures. Since total C constitutes all carbohydrates, including mannitol and phlorotannins (Amsler 2008 and references therein) but no correlation with mannitol was found, phlorotannins most likely make up a large proportion in the increase of total C and thus C:N.

Phlorotannins were not affected by the temperature-amplitude treatments and did not increase significantly at suboptimal high temperatures as was shown in brown algae before (Simonson et al. 2015a, Flores-Molina et al. 2016), but they increased with absolute temperature and strong local differentiations were found. Phlorotannin content is strongly connected with wound healing and light environments (Amsler 2008 and references therein). Induction and acclimation occur within a few hours to days (Hammerstrom et al. 1998, Cruces et al. 2013), meaning that potential impacts of sampling and *in situ* radiation were extenuated by the experimental design. Significantly fewer phlorotannins were measured in the samples from Spitsbergen and the concentrations were comparable to the ones measured in other

Arctic *S. latissima* (Diehl & Bischof 2021). These observations apparently argue for temperature acclimation in phlorotannins of *S. latissima* to some extent, even though samples from Bergen revealed the highest content.

We could not verify the proposed strategy of Arctic *S. latissima* to constantly store high mannitol concentrations as acclimation to frequent environmental variations (Diehl & Bischof 2021), even though mannitol content slightly decreased at lower latitudes at the end of the temperature-amplitude experiment. In their experiment, Diehl & Bischof (2021) mimicked Arctic summer conditions and maintained the samples under constant 24 h of light exposure. For our current study, we kept all samples at a 16:8-h-light:dark rhythm to eliminate potential light effects at the different latitudes, which might have influenced the synthesis of mannitol.

Phenotypic plasticity or local adaptation?

It is an interesting question in terms of conservation ecology whether acclimation mechanisms of species are based on phenotypic plasticity (the ability of individual genotypes to produce different phenotypes when exposed to different conditions, short-term) or on local adaptation (the process of genetic change that accumulates over many generations, long-term) (Morgan-Kiss et al. 2006, Pigliucci et al. 2006). Long-term exposure to different selective environmental pressures can result in the development of distinct ecotypes (Nicotra et al. 2010), although locally adapted ecotypes might also exhibit high phenotypic plasticity (de Jong 2005). As reported for *Laminaria digitata*, the emerge of ecotypes in kelps can be very subtle and with high variation in plasticity (Liesner et al. 2020a, Martins et al. 2020). Nevertheless, different populations are often treated as a single homogenous physiological unit, and within-species variations to environmental factors are overlooked (Reed et al. 2011, Bennett et al. 2019).

Saccharina latissima is commonly found along the entire European Atlantic coast from polar regions down to a latitude of ~ 40°N (Araújo et al. 2016). With its widespread distribution and the corresponding variability in abiotic drivers, both phenotypic plasticity and local adaptation have been reported and their partition appeared to be very complex (e.g. Lüning 1975, Müller et al. 2008, Olischläger et al. 2014, 2017). For instance, while Bolton & Lüning (1982) did not find thermal ecotypes with respect to growth or survival and proposed that *S. latissima* rather expresses high phenotypic plasticity, Gerard & Du Bois (1988) report different thermal tolerances in two USA populations. Accordingly, it remains unclear whether *S. latissima* exhibits thermal ecotypes or not.

Clear genetic differentiation between populations is prerequisites for the potential occurrence of local adaptation and ecotypes along the distribution range of *S. latissima* (Guzinski et al. 2020) since the existence of distinct ecotypes entails genetic adaptations to local temperature conditions (Conner & Hartl 2004). Guzinski et al. (2020) investigated the genetic diversity of European *S. latissima* and found a high degree of inter-population differentiation and no genetic connectivity but no significant variation in genetic diversity with latitude.

To answer the question of the existence of thermal ecotypes in *S. latissima*, common garden experiments would be required, comparable to Gerard & Du Bois (1988), Liesner et al. (2020*a*) or Martins et al. (2020). However, our approach focuses on the performance of *S. latissima* across latitudes to relative temperature increases. The existence of restricted thermal ecotypes would provoke equal responses to the same relative temperature increase (Δ+2 – 6 °C) in the different populations. Our data clearly show that differentiations between populations were not reflected in response patterns to the temperature amplitudes. While samples from locations with high mean summer SST suffered under increased temperatures, the northern samples with lower temperatures were unaffected or even benefitted slightly. Since exclusively *S. latissima* populations with high mean summer SST were negatively affected by increasing temperatures, we can conclude that absolute temperatures delimit the physiological performance of *S. latissima* and not relative temperature anomaly in summer. Only when reaching sublethal to lethal temperature thresholds, *S. latissima* revealed thermal stress behavior through several traits. Thus, even if subtle thermal ecotypes exist within *S. latissima* in Europe as reported for *L. digitata* (Liesner et al. 2020a, Martins et al. 2020), the adaptations to different temperature ranges would not have an ecological effect on sporophytes under Δ+2 – 6 °C MHW scenarios in summer.

In our study, physiological and biochemical traits exhibited different plasticity to temperatures. Selection can act on multiple phenotypic traits and consequently affects the rate of evolution (Reed et al. 2011). High plasticity may support a wide biogeographical distribution, potentially leading to local adaptations as a result of long exposition to different local selective pressures. Under continued environmental pressure, this possibly provoked the evolvement of thermal ecotypes on an even larger geographical scale (Nicotra et al. 2010), but fast adaptation to temperature is unlikely to have taken place in North Atlantic species such as the genus *Laminaria* (Wiencke et al. 1994).

Implications for the population biology of Saccharina latissima *in Europe*

Even though *S. latissima* tolerates wide amplitudes of physico-chemical drivers (reviewed by Bartsch et al. 2008), strong declines and changes in biomass are reported from several regions in Europe (Pehlke & Bartsch 2008, Moy & Christie 2012, Casado-Amezúa et al. 2019, Filbee-Dexter et al. 2020). Heat tolerance and the capacity for acclimation may differ between genetic strains, thus populations (Clark et al. 2013, Nepper-Davidsen et al. 2019). Our study shows that subtle thermal ecotypes play a subordinated role in coping with short-term ocean warming. Despite the existence of potential thermal ecotypes (Müller et al. 2009 and references therein) and the low genetic connectivity between *S. latissima* populations along the European coastline (Guzinski et al. 2020), the overall responses to thermal stress under realistic heatwave-like events (i.e. Δ+2 – 6 °C) were not affected. Local MHWs in summer only pose stress to *S. latissima* in regions with higher mean summer sea surface temperatures. Thus, variable local temperature thresholds for survival, as present in thermal ecotypes, do not contribute to mortality in European *S. latissima*, as suggested for other kelps (Wernberg et al. 2010, King et al. 2018). Nevertheless, we found indications to differentiation in responses to high temperatures between the populations in Helgoland and Locmariaquer, which should be examined in more detail.

We suggest that the environmental background and seasonal temperature history is the crucial factor in temperature tolerance and distribution traits of *S. latissima*. Growth temperature is known to affect the photosynthetic performance of *S. latissima* (Davison 1987, Davison & Davison 1987, Davison et al. 1991), and the importance of temperature history on thermal plasticity of kelps was recently published by Liesner et al. (2020b), who investigated the effect of temperatures on the development of juvenile *L. digitata*. They provided evidence for non-genetic carry-over and cross-generational effects and reported a stimulating effect of cold temperatures on several physiological and biochemical responses. Rapid and intense ocean warming in the winter season, which was already detected and is predicted to further increase in the near future (Maturilli et al. 2013, IPCC 2019b), might therefore particularly affect thermal plasticity and stress resilience to high temperatures also in summer and hence contribute to kelp reduction. By exceeding optimum temperatures, reversible physiological responses manifest (Davison et al. 1991, Wernberg et al. 2016), and specimens might be less resilient to other stressors or less competitive against other algae (Wernberg et al. 2010, Moy & Christie 2012, Andersen et al. 2013). Additionally, the interaction with other abiotic drivers

such as nutrients, irradiance or salinity were shown to affect habitat-specific temperature tolerances in *S. latissima* (Machalek et al. 1996, Gerard 1997a, Diehl & Bischof 2021, Monteiro et al. 2021). Summarizing, summer MHWs alone do not cause reductions in European kelp populations across latitudes, instead, declines are caused by an interplay of many different factors.

Acknowledgements

Abiotic temperature data used in this paper were produced with the Giovanni online data system, developed and maintained by the NASA GES DISC. Sampling in France was conducted in accordance with the French legislation on the Access to Genetic Resources and Benefit-Sharing. We also acknowledge the MODIS mission scientists and associated NASA personnel for the production of the data used in this research effort. This study has been conducted at the Alfred Wegener Institute for Polar and Marine Research (AWI), Bremerhaven and at the AWIPEV Research Station in Ny-Ålesund, Svalbard. The authors are grateful to the station staff of AWIPEV for support and logistics and to the scientific diving teams on Spitsbergen and Helgoland for sampling. The authors thank A. Wagner (AWI) for sampling support on Helgoland and his support in the setting up the experiments, and also J. Müller (University of Rostock) for running the C:N analyzer. B. Meyer-Schlosser (University of Bremen) supported sampling and pigment analyses. This study was further supported by the 2015 – 2016 BiodiversERsA COFUND call for research proposals (program MARFOR), with the national funder German Research Foundation (DFG; grant no. VA 105/25-1).

References

Acker JG, Leptoukh G (2007) Giovanni Satellite: Online analysis enhances use of NASA Earth Science Data. *Eos, Trans Am Geophys Union* 88:14–17.

Adey WH, Steneck RS (2001) Thermogeography over time creates biogeographic regions: A temperature/space/time-integrated model and an abundance-weighted test for benthic marine algae. *J Phycol* 37:677–698.

Amsler CD (2008) Algal chemical ecology. Amsler CD (ed) Springer-Verlag Berlin Heidelberg.

Andersen G, Pedersen M, Nielsen S (2013) Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima* (Laminariales, Phaeophyceae). *J Phycol* 49:689–700.

Arafeh-Dalmau N, Schoenman DS, Montaño-Moctezuma G, Micheli F, Rogers-Bennett L, Olguin-Jacobson C, Possingham HP (2020) Marine heat waves threaten kelp forests. *Science* 367:635.

Araújo RM, Assis J, Aguillar R, Airoldi L, Bárbara I, Bartsch I, Bekkby T, Christie H, Davoult D, Derrien-Courtel S, Fernandez C, Fredriksen S, Gevaert F, Gundersen H, Le Gal A, Lévêque L, Mieszkowska N, Norderhaug KM, Oliveira P, Puente A, Rico JM, Rinde E, Schubert H, Strain EM, Valero M, Viard F, Sousa-Pinto I (2016) Status, trends and drivers of kelp forests in Europe: an expert assessment. *Biodivers Conserv* 25:1319–1348.

Assis J, Araújo MB, Serrão EA (2018) Projected climate changes threaten ancient refugia of kelp forests in the North Atlantic. *Glob Change Biol* 24:e55–e66.

Atkinson MJ, Smith SV (1983) C:N:P ratios of benthic marine plants. *Limnol Oceanogr* 28:568–574.

Bartsch I, Vogt J, Pehlke C, Hanelt D (2013) Prevailing sea surface temperatures inhibit summer reproduction of the kelp *Laminaria digitata* at Helgoland (North Sea). *J Phycol* 49:1061–1073.

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J (2008) The genus *Laminaria sensu lato*: Recent insights and developments. *Eur J Phycol* 43:1–86.

Bekkby T, Moy FE (2011) Developing spatial models of sugar kelp (*Saccharina latissima*) potential distribution under natural conditions and areas of its disappearance in Skagerrak. *Estuar Coast Shelf Sci* 95:477–483.

Bennett S, Duarte CM, Marbà N, Wernberg T (2019) Integrating within-species variation in thermal physiology into climate change ecology. *Philos Trans R Soc B* 374:20180550.

Bennett S, Wernberg T, Joy BA, De Bettignies T, Campbell AH (2015) Central and rear-edge populations can be equally vulnerable to warming. *Nat Commun* 6:10280.

Bolton JJ, Lüning K (1982) Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. *Mar Biol* 66:89–94.

Burdett HL, Wright H, Smale DA (2019) Photophysiological responses of canopy-forming kelp species to short-term acute warming. *Front Mar Sci* 6:516.

Casado-Amezúa P, Araújo R, Bárbara I, Bermejo R, Borja, Díez I, Fernández C, Gorostiaga JM, Guinda X, Hernández I, Juanes JA, Peña V, Peteiro C, Puente A, Quintana I, Tuya F, Viejo RM, Altamirano M, Gallardo T, Martínez B (2019) Distributional shifts of canopy-forming seaweeds from the Atlantic coast of southern Europe. *Biodivers Conserv* 28:1151–1172.

Clark JS, Poore AGB, Ralph PJ, Doblin MA (2013) Potential for adaptation in response to thermal stress in an intertidal macroalga. *J Phycol* 49:630–639.

Cohen J (1988) Statistical power analysis for the behavioral sciences, Second Edi. Lawrence Erlbaum Associates, United States of America, New York.

Collén J, Davison IR (2001) Seasonality and thermal acclimation of reactive oxygen metabolism in in *Fucus versiculosus* (Phaeophyceae). *J Phycol* 37:474–481.

Colombo-Pallotta MF, García-Mendoza E, Ladah LB (2006) Photosynthetic performance, light absorption, and pigment composition of *Macrocystis pyrifera* (Laminariales, Phaeophyceae) blade from different depths. *J Phycol* 42:1225–1234.

Conner JK, Hartl D. (2004) A primer of ecological genetics. Sinauer Associates Incorporated, Sunderland, USA.

Cruces E, Huovinen P, Gómez I (2013) Interactive effects of UV radiation and enhanced temperature on photosynthesis, phlorotannin induction and antioxidant activities of two sub-Antarctic brown algae. *Mar Biol* 160:1–13.

Cruces E, Huovinen P, Gómez I (2012) Phlorotannin and antioxidant responses upon short-term exposure to UV radiation and elevated temperature in three South Pacific kelps. *Photochem Photobiol* 88:58–66.

Davison IR (1987) Adaptation of photosynthesis in *Laminaria saccharina* (Phaeophyta) to changes in growth temperature. *J Phycol* 23:273–283.

Davison IR, Davison JO (1987) The effect of growth temperature on enzyme activities in the brown alga *Laminaria saccharina*. *Br Phycol J* 22:77–87.

Davison IR, Greene RM, Podolak EJ (1991) Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina*. *Mar Biol* 110:449–454.

Dayton PK (1985) Ecology of kelp communities. *Annu Rev Ecol Syst* 16:215–245.

Diehl N, Bischof K (2021) Coping with a changing Arctic: mechanisms of acclimation in the brown seaweed *Saccharina latissima* from Spitsbergen. *Mar Ecol Prog Ser* 657:43–57.

Diehl N, Karsten U, Bischof K (2020) Impacts of combined temperature and salinity stress on the endemic Arctic brown seaweed *Laminaria solidungula* J. Agardh. *Polar Biol* 43:647–656.

Dring MJ (2006) Stress resistance and disease resistance in seaweeds: the role of reactive oxygen metabolism. *Adv Bot Res* 43:175–207.

Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). *Mar Biol* 126:183–191.

Dunton KH (1985) Growth of dark-exposed *Laminaria saccharina* (L.) Lamour. and *Laminaria solidungula* J. Ag. (Laminariales : Phaeophyta) in the Alaskan Beaufort Sea. *J Exp Mar Bio Ecol* 94:181–189.

Fernandes F, Barbosa M, Oliveira AP, Azevedo IC, Sousa-Pinto I, Valentão P, Andrade PB (2016) The pigments of kelps (Ochrophyta) as part of the flexible response to highly variable marine environments. *J Appl Phycol* 28:3689–3696.

Fernández PA, Gaitán-Espitia JD, Leal PP, Schmid M, Revill AT, Hurd CL (2020) Nitrogen sufficiency enhances thermal tolerance in habitat-forming kelp: implications for acclimation under thermal stress. *Sci Rep* 10:3186.

Filbee-Dexter K, Wernberg T, Grace SP, Thormar J, Fredriksen S, Narvaez CN, Feehan CJ, Norderhaug KM (2020) Marine heatwaves and the collapse of marginal North Atlantic kelp forests. *Sci Rep* 10:13388.

Flores-Molina MR, Rautenberger R, Muñoz P, Huovinen P, Gómez I (2016) Stress tolerance of the endemic Antarctic brown alga *Desmarestia anceps* to UV radiation and temperature is mediated by high concentrations of phlorotannins. *Photochem Photobiol* 92:455–466.

Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgol Meeresunters* 34:15–29.

Franke K (2019) Performance of different life cycle stages of the Arctic kelps *Laminaria digitata* and *Saccharina nigripes* along temperature gradients. Master Thesis. University of Bremen.

Frölicher TL, Fischer EM, Gruber N (2018) Marine heatwaves under global warming. *Nature* 560:360–364.

Gerard VA (1997a) Environmental stress during early development of kelp sporophytes (*Laminaria saccharina*): How long do effects persist? *J Appl Phycol* 9:5–9.

Gerard VA (1997b) The role of nitrogen nutrition in high-temperature tolerance of the kelp, *Laminaria saccharina* (Chromophyta). *J Phycol* 33:800–810.

Gerard VA, Du Bois KR (1988) Temperature ecotypes near the southern boundary of the kelp *Laminaria saccharina*. *Mar Biol* 97:575–580.

Gevaert F, Davoult D, Creach A, Kling R, Janquin M-A, Seuront L, Lemoine Y (2001) Carbon and nitrogen content of *Laminaria saccharina* in the eastern English Channel: Biometrics and seasonal variations. *J Mar Biol Assoc U K* 81:727–734.

Goss R, Jakob T (2010) Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynth Res* 106:103–122.

Graiff A, Liesner D, Karsten U, Bartsch I (2015) Temperature tolerance of western Baltic Sea *Fucus vesiculosus* – growth, photosynthesis and survival. *J Exp Mar Bio Ecol* 471:8–16.

Guzinski J, Ruggeri P, Ballenghien M, Mauger S, Jacquemin B, Jollivet C, Coudret J, Jaugeon L, Destombe C, Valero M (2020) Seascape genomics of the sugar kelp *Saccharina latissima* along the north eastern Atlantic latitudinal gradient. *Genes* 11:1503.

Hammerstrom K, Dethier MN, Duggin DO (1998) Rapid phlorotannin induction and relaxation in five Washington kelps. *Mar Ecol Prog Ser* 165:293–305.

Hanelt D, Wiencke C, Karsten U, Nultsch W (1997) Photoinhibition and recovery after high light stress in different developmental and life-history stages of *Laminaria saccharina* (Phaeophyta). *J Phycol* 33:387–395.

Harris RMB, Beaumont LJ, Vance TR, Tozer CR, Remenyi TA, Perkins-Kirkpatrick SE, Mitchell PJ, Nicotra AB, McGregor S, Andrew NR, Letnic M, Kearney MR, Wernberg T, Hutley LB, Chambers LE, Fletcher MS, Keatley MR, Woodward CA, Williamson G, Duke NC, Bowman DMJS (2018) Biological responses to the press and pulse of climate trends and extreme events. *Nat Clim Change* 8:579–587.

Heinrich S, Valentin K, Frickenhaus S, Wiencke C (2016) Origin matters – Comparative transcriptomics in *Saccharina latissima* (Phaeophyceae). *J Exp Mar Bio Ecol* 476:22–30.

Hobday AJ, Alexander L V., Perkins SE, Smale DA, Straub SC, Oliver ECJ, Benthuysen JA, Burrows MT, Donat MG, Feng M, Holbrook NJ, Moore PJ, Scannell HA, Sen Gupta A, Wernberg T (2016) A hierarchical approach to defining marine heatwaves. *Prog Oceanogr* 141:227–238.

Iñiguez C, Carmona R, Lorenzo MR, Niell FX, Wiencke C, Gordillo FJL (2016) Increased temperature, rather than elevated CO_2, modulates the carbon assimilation of the Arctic kelps *Saccharina latissima* and *Laminaria solidungula*. *Mar Biol* 163:248.

IPCC (2019a) Summary for policymakers. In: *IPCC Special report on the ocean and cryosphere in a changing climate*. Pörtner H-O, Roberts DC, Masson-Delmotte V, Zhai P, Tignor M, Poloczanska E, Mintenbeck K, Alegría A, Nicolai M, Okem A, Petzold J, Rama B, Weyer NM (eds).

IPCC (2019b) Summary for Policymakers. In: *Climate change and land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems*. Shukla PR, Skea J, Calvo Buendia E, Masson-Delmotte V, Pörtner H-O, Roberts DC, Zhai P, Slade R, Connors S, van Diemen R, Ferrat M, Haughey E, Luz S, Neogi S, Pathak M, Petzold J, Portugal Pereira J, Vyas P, Huntley E, Kissick K, Belkacemi M, Malley J (eds).

Iwamoto K, Shiraiwa Y (2005) Salt-regulated mannitol metabolism in algae. *Mar Biotechnol* 7:407–415.

Jennings DB, Ehrenshaft M, Mason Pharr D, Williamson JD (1998) Roles for mannitol and mannitol dehydrogenase in active oxygen-mediated plant defense. *Proc Natl Acad Sci U S A* 95:15129–15133.

de Jong G (2005) Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. *New Phytol* 166:101–118.

Jueterbock A, Kollias S, Smolina I, Fernandes JMO, Coyer JA, Olsen JL, Hoarau G (2014) Thermal stress resistance of the brown alga *Fucus serratus* along the North-Atlantic coast: Acclimatization potential to climate change. *Mar Genomics* 13:27–36.

Karsten U (2007) Salinity tolerance of Arctic kelps from Spitsbergen. *Phycol Res* 55:257–262.

Karsten U, Thomas DN, Weykam G, Daniel C, Kirst GO (1991) A simple and rapid method for extraction and separation of low molecular weight carbohydrates from macroalgae using high-performance liquid chromatography, II. Intracellular inorganic ions and organic compounds. *Plant Physiol Biochem* 29:373–378.

Kelly MW, Sanford E, Grosberg RK (2012) Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proc R Soc B Biol Sci* 279:349–356.

King NG, Mckeown NJ, Smale DA, Moore PJ (2018) The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. *Ecography* 41:1469–1484.

Koch K, Thiel M, Tellier F, Hagen W, Graeve M, Tala F, Laeseke P, Bischof K (2015) Species separation within the *Lessonia nigrescens* complex (Phaeophyceae, Laminariales) is mirrored by ecophysiological traits. *Bot Mar* 58:81–92.

Li H, Monteiro C, Heinrich S, Bartsch I, Valentin K, Harms L, Glöckner G, Corre E, Bischof K (2020) Responses of the kelp *Saccharina latissima* (Phaeophyceae) to the warming Arctic: From physiology to transcriptomics. *Physiol Plant* 168:5–26.

Liesner D, Fouqueau L, Valero M, Roleda MY, Pearson GA, Bischof K, Valentin K, Bartsch I (2020a) Heat stress responses and population genetics of the kelp *Laminaria digitata* (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations. *Ecol Evol* 10:9144–9177.

Liesner D, Shama LNS, Diehl N, Valentin K, Bartsch I (2020b) Thermal plasticity of the kelp *Laminaria digitata* (Phaeophyceae) across life cycle stages reveals the importance of cold seasons for marine forests. *Front Mar Sci* 7:456.

Lourenço CR, Zardi GI, McQuaid CD, Serrão EA, Pearson GA, Jacinto R, Nicastro KR (2016) Upwelling areas as climate change refugia for the distribution and genetic diversity of a marine macroalga. *J Biogeogr* 43:1595–1607.

Lüning K (1979) Growth strategies of three *Laminaria* species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). *Mar Ecol Prog Ser* 1:195–207.

Lüning K (1975) Kreuzungsexperimente an *Laminaria saccharina* von Helgoland und von der Isle of Man. *Helgol Wiss Meeresunters* 27:108–114.

Lüning K (1988) Photoperiodic control of sorus formation in the brown alga *Laminaria saccharina*. *Mar Ecol Prog Ser* 45:137–144.

Lüning K (1990) Seaweeds – Their environment, biogeography, and ecophysiology. Yarish C, Kirkman H (eds) John Wiley & Sons, Inc.

Lüning K (1984) Temperature tolerance and biogeography of seaweeds: The marine algal flora of Helgoland (North Sea) as an example. *Helgol Meeresunters* 28:305–317.

Machalek K, Davison R, Falkwoski P (1996) Thermal acclimation and photoacclimation of photosynthesis in the brown alga *Laminaria saccharina*. *Plant Cell Environ* 19:1005–1016.

Martins N, Pearson GA, Bernard J, Serrão EA, Bartsch I (2020) Thermal traits for reproduction and recruitment differ between Arctic and Atlantic kelp *Laminaria digitata*. *PLoS One* 15:e0235388.

Maturilli M, Herber A, König-Langlo G (2013) Climatology and time series of surface meteorology in Ny-Ålesund, Svalbard. *Earth Syst Sci Data* 5:155–163.

Meehl GA, Tebaldi C (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* 305:994–997.

Monteiro C, Li H, Diehl N, Collén J, Heinrich S, Bischof K, Bartsch I (2021) Modulation of physiological performance by temperature and salinity in the sugar kelp *Saccharina latissima*. *Phycol Res* 69:48–57.

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Huner NPA (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiol Mol Biol Rev* 70:222–252.

Moy FE, Christie H (2012) Large-scale shift from sugar kelp (*Saccharina latissima*) to ephemeral algae along the south and west coast of Norway. *Mar Biol Res* 8:309–321.

Müller R, Laepple T, Bartsch I, Wiencke C (2009) Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Bot Mar* 52:617–638.

Müller R, Wiencke C, Bischof K (2008) Interactive effects of UV radiation and temperature on microstages of Laminariales (Phaeophyceae) from the Arctic and North Sea. *Clim Res* 37:203–213.

Nepper-Davidsen J, Andersen D, Pedersen M (2019) Exposure to simulated heatwave scenarios causes long-term reductions in performance in *Saccharina latissima*. *Mar Ecol Prog Ser* 630:25–39.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. *Trends Plant Sci* 15:684–692.

Olischläger M, Iñiguez C, Gordillo FJL, Wiencke C (2014) Biochemical composition of temperate and Arctic populations of *Saccharina latissima* after exposure to increased pCO_2 and temperature reveals ecotypic variation. *Planta* 240:1213–1224.

Olischläger M, Iñiguez C, Koch K, Wiencke C, Gordillo FJL (2017) Increased pCO_2 and temperature reveal ecotypic differences in growth and photosynthetic performance of temperate and Arctic populations of *Saccharina latissima*. *Planta* 245:119–136.

Oliver ECJ, Donat MG, Burrows MT, Moore PJ, Smale DA, Alexander L V., Benthuysen JA, Feng M, Sen Gupta A, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell HA, Straub SC, Wernberg T (2018) Longer and more frequent marine heatwaves over the past century. *Nat Commun* 9:1324.

Pehlke C, Bartsch I (2008) Changes in depth distribution and biomass of sublittoral seaweeds at Helgoland (North Sea) between 1970 and 2005. *Clim Res* 37:135–147.

Pereira TR, Engelen AH, Pearson GA, Valero M, Serrão EA (2015) Response of kelps from different latitudes to consecutive heat shock. *J Exp Mar Bio Ecol* 463:57–62.

Pigliucci M, Murren CJ, Schlichting CD (2006) Phenotypic plasticity and evolution by genetic assimilation. *J Exp Biol* 209:2362–2367.

Provasoli L (1968) Media and prospects for the cultivation of marine algae. In: *Cultures and collections of algae*. *Proceedings of the Japanese Conference Hakone*. Japanese Society of Plant Physiology. Watanabe A, Hattori A (eds) Tokyo, p 63–75.

QGIS Development Team (2019) QGIS 3.8.2-Zanzibar software. http://qgis.osgeo.org.

Rautenberger R, Bischof K (2006) Impact of temperature on UV-susceptibility of two *Ulva* (Chlorophyta) species from Antarctic and Subantarctic regions. *Polar Biol* 29:988–996.

Reed RH, Davison IR, Chudek JA, Foster R (1985) The osmotic role of mannitol in the Phaeophyta: an appraisal. *Phycologia* 24:35–47.

Reed TE, Schindler DE, Waples RS (2011) Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conserv Biol* 25:56–63.

Reich PB, Sendall KM, Rice K, Rich RL, Stefanski A, Hobbie SE, Montgomery RA (2015) Geographic range predicts photosynthetic and growth response to warming in co-occurring tree species. *Nat Clim Change* 5:148–152.

Roleda MY, Hurd CL (2019) Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. *Phycologia* 58:552–562.

Saha M, Barboza FR, Somerfield PJ, Al-Janabi B, Beck M, Brakel J, Ito M, Pansch C, Nascimento-Schulze JC, Jakobsson Thor S, Weinberger F, Sawall Y (2020) Response of foundation macrophytes to near-natural simulated marine heatwaves. *Glob Change Biol* 26:417–430.

Shen B, Jensen RG, Bohnert HJ (1997) Mannitol protects against oxidation by hydroxyl radicals. *Plant Physiol* 115:527–532.

Simonson EJ, Metaxas A, Scheibling RE (2015a) Kelp in hot water: II. Effects of warming seawater temperature on kelp quality as a food source and settlement substrate. *Mar Ecol Prog Ser* 537:105–119.

Simonson EJ, Scheibling RE, Metaxas A (2015b) Kelp in hot water: I. Warming seawater temperature induces weakening and loss of kelp tissue. *Mar Ecol Prog Ser* 537:89–104.

Sjøtun K (1993) Seasonal lamina growth in two age groups of *Laminaria saccharina* (L.) Lamour. in Western Norway. *Bot Mar* 36:433–442.

Smale DA, Wernberg T, Oliver ECJ, Thomsen M, Harvey BP, Straub SC, Burrows MT, Alexander LV, Benthuysen JA, Donat MG, Feng M, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell HA, Sen Gupta A, Payne BL, Moore PJ (2019) Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nat Clim Change* 9:306–312.

Springer K, Lütz C, Lütz-Meindl U, Wendt A, Bischof K (2017) Hyposaline conditions affect UV susceptibility in the Arctic kelp *Alaria esculenta* (Phaeophyceae). *Phycologia* 56:675–685.

Spurkland T, Iken K (2011) Kelp bed dynamics in estuarine environments in subarctic Alaska. *J Coast Res* 27:133–143.

Spurkland T, Iken K (2012) Seasonal growth patterns of *Saccharina latissima* (Phaeophyceae, Ochrophyta) in a glacially-influenced subarctic estuary. *Phycol Res* 60:261–275.

Staehr PA, Wernberg T (2009) Physiological responses of *Ecklonia radiata* (Laminariales) to a latitudinal gradient in ocean temperature. *J Phycol* 45:91–99.

Sunday JM, Pecl GT, Frusher S, Hobday AJ, Hill N, Holbrook NJ, Edgar GJ, Stuart-Smith R, Barrett N, Wernberg T, Watson RA, Smale DA, Fulton EA, Slawinski D, Feng M, Radford BT, Thompson PA, Bates AE (2015) Species traits and climate velocity explain geographic range shifts in an ocean-warming hotspot. *Ecol Lett* 18:944–953.

Tatewaki M (1966) Formation of a crustaceous sporophyte with unilocular sporangia in *Scytosiphon lomentaria*. *Phycologia* 6:62–66.

Terblanche JS, Deere JA, Clusella-trullas S, Janion C, Chown SL (2007) Critical thermal limits depend on methodological context. *Proc R Soc B* 274:2935–2942.

Thomsen MS, Mondardini L, Alestra T, Gerrity S, Tait L, South PM, Lilley SA, Schiel DR (2019) Local extinction of bull kelp (*Durvillaea* spp.) due to a marine heatwave. *Front Mar Sci* 6:1–10.

Tuya F, Cacabelos E, Duarte P, Jacinto D, Castro JJ, Silva T, Bertocci I, Franco JN, Arenas F, Coca J, Wernberg T (2012) Patterns of landscape and assemblage structure along a latitudinal gradient in ocean climate. *Mar Ecol Prog Ser* 466:9–19.

Verardo DJ, Froelich PN, McIntyre A (1990) Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 Analyzer. *Deep Res* 37:157–165.

Voerman SE, Llera E, Rico JM (2013) Climate driven changes in subtidal kelp forest communities in NW Spain. *Mar Environ Res* 90:119–127.

Wang Y, Yu Q, Tang X, Wang L (2009) Calcium pretreatment increases thermotolerance of *Laminaria japonica* sporophytes. *Prog Nat Sci* 19:435–442.

Wernberg T, Bennett S, Babcock RC, Bettignies T De, Cure K, Depczynski M, Dufois F, Fromont J, Fulton CJ, Hovey RK, Harvey ES, Holmes TH, Kendrick GA, Radford B, Santana-Garcon J, Saunders BJ, Smale DA, Thomsen MS, Tuckett CA, Tuya F (2015) Climate-driven regime shift of a temperate marine ecosystem. *Science* 353:169–172.

Wernberg T, de Bettignies T, Joy BA, Finnegan PM (2016) Physiological responses of habitat-forming seaweeds to increasing temperatures. *Limnol Oceanogr* 61:2180–2190.

Wernberg T, Thomsen MS, Tuya F, Kendrick GA, Staehr PA, Toohey BD (2010) Decreasing resilience of kelp beds along a latitudinal temperature gradient: Potential implications for a warmer future. *Ecol Lett* 13:685–694.

Wiencke C, Bischof K (2012) Seaweed Biology – Novel insights into ecophysiology, ecology and utilization. Springer, Heidelberg New York Dodrecht London.

Wiencke C, Bischoff B, Bartsch I, Peters AF, Breeman AM (1994) Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. *Bot Mar* 37:247–260.

Winters G, Nelle P, Fricke B, Rauch G, Reusch TBH (2011) Effects of a simulated heat wave on photophysiology and gene expression of high- and low-latitude populations of *Zostera marina*. *Mar Ecol Prog Ser* 435:83–95.

Wright SW, Jeffrey SW, Mantoura RFC, Llewelly CA, Bjørnland T, Repeta D, Welschmeyer N (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar Ecol Prog Ser* 77:183–196.

Yancey PH (2005) Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J Exp Biol* 208:2819–2830.

Zhou B, Tang X, Wang Y (2010) Salicylic acid and heat acclimation pretreatment protects *Laminaria japonica* sporophyte (Phaeophyceae) from heat stress. *Chinese J Oceanol Limnol* 28:924–932.

Supplement

Material and methods

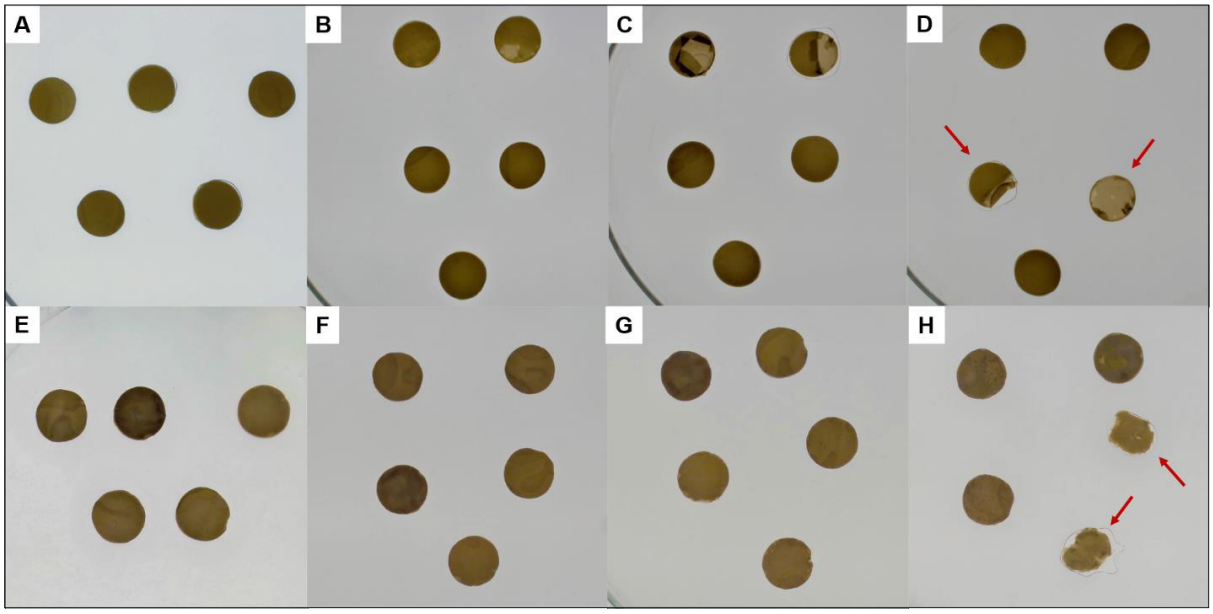


Fig. S2.1: Stages of decomposition of *Saccharina latissima* during the temperature-amplitude experiment on days 2, 4, 6 and 8. Red arrows mark discs which were considered 'dead' at the end of the experiment. **A – D**: Helgoland. **E – H**: Locmariaquer.

Biochemical data

Table S2.1: Absolute concentration of fucoxanthin, chlorophyll *c*2, violaxanthin, antheraxanthin, zeaxanthin, total carbon and total nitrogen of *Saccharina latissima* across latitudes in Europe after the temperature-amplitude experiment. Experimental set-up: temperature-amplitude treatment (treatment; Δ°C) and respective absolute temperature (abs. temp.; °C). Values are means ± SD (n = 4). DW = dry weight.

population	treatment (Δ°C)	abs. temp. (°C)	fucoxanthin (mg g^{-1} DW)	chlorophyll *c*2 (mg g^{-1} DW)	violaxanthin (µg g^{-1} DW)	antheraxanthin (µg g^{-1} DW)	zeaxanthin (µg g^{-1} DW)	total carbon (mg g^{-1} DW)	total nitrogen (mg g^{-1} DW)
Spitsbergen	±0	6	0.411 ± 0.056	0.157 ± 0.022	61.77 ± 14.62	2.58 ± 1.43	2.24 ± 1.86	275.42 ± 35.12	18.63 ± 1.96
	+2	8	0.478 ± 0.112	0.195 ± 0.050	80.78 ± 24.72	4.70 ± 3.64	2.53 ± 2.34	237.72 ± 47.33	17.54 ± 5.36
	+4	10	0.421 ± 0.243	0.172 ± 0.112	66.51 ± 46.09	2.08 ± 1.11	1.01 ± 0.54	291.33 ± 21.46	22.87 ± 1.89
	+6	12	0.511 ± 0.115	0.194 ± 0.023	67.70 ± 7.76	2.28 ± 0.59	1.17 ± 0.81	260.31 ± 40.07	18.86 ± 1.42
Bodø	±0	12	1.038 ± 0.168	0.360 ± 0.043	45.34 ± 6.70	1.86 ± 0.40	2.33 ± 0.85	337.40 ± 1.31	21.94 ± 1.26
	+2	14	1.007 ± 0.163	0.362 ± 0.050	53.43 ± 4.38	2.47 ± 0.99	2.33 ± 0.48	316.73 ± 7.50	21.27 ± 1.66
	+4	16	0.863 ± 0.126	0.308 ± 0.055	38.50 ± 14.00	2.37 ± 0.74	1.59 ± 0.38	322.75 ± 16.23	21.46 ± 0.62
	+6	18	0.970 ± 0.158	0.324 ± 0.060	51.74 ± 7.28	2.35 ± 0.51	1.01 ± 0.23	303.78 ± 16.49	21.36 ± 2.16
Bergen	±0	16	0.689 ± 0.117	0.223 ± 0.028	77.63 ± 23.81	3.98 ± 0.93	0.96 ± 0.76	289.54 ± 21.04	23.88 ± 3.31
	+2	18	0.714 ± 0.153	0.229 ± 0.041	79.79 ± 24.36	3.37 ± 1.26	1.30 ± 0.24	340.02 ± 39.16	30.76 ± 4.65
	+4	20	0.618 ± 0.054	0.188 ± 0.017	76.87 ± 20.64	4.67 ± 0.19	1.19 ± 0.26	290.22 ± 43.82	21.79 ± 2.11
	+6	22	0.707 ± 0.140	0.210 ± 0.043	88.52 ± 26.64	8.31 ± 1.81	1.83 ± 0.78	306.87 ± 17.15	21.04 ±1.34
Helgoland	±0	18	0.705 ± 0.147	0.215 ± 0.039	61.60 ± 12.80	4.06 ± 3.31	2.14 ± 2.65	308.78 ± 18.82	16.28 ± 2.91
	+2	20	0.651 ± 0.096	0.197 ± 0.027	59.05 ± 7.62	3.71 ± 0.95	0.76 ± 0.34	304.31 ± 21.87	16.38 ± 2.76
	+4	22	0.657 ± 0.063	0.186 ± 0.019	59.90 ± 15.23	6.60 ± 0.66	9.26 ± 15.20	297.35 ± 13.97	16.93 ± 3.11
	+6	24	0.497 ± 0.131	0.128 ± 0.035	28.32 ± 7.38	20.73 ± 6.68	59.25 ± 23.23	300.08 ± 22.47	15.47 ± 1.17
Locmariaquer	±0	19	0.412 ± 0.110	0.134 ± 0.047	53.03 ± 17.66	1.09 ± 0.22	0.67 ± 0.32	321.53 ± 36.20	23.61 ± 1.53
	+2	21	0.338 ± 0.190	0.117 ± 0.066	48.69 ± 27.66	1.14 ± 0.72	0.85 ± 0.53	319.04 ± 30.88	20.43 ± 2.39
	+4	23	0.425 ± 0.150	0.134 ± 0.055	57.81 ± 13.64	1.94 ± 0.45	1.27 ± 0.56	327.98 ± 28.19	20.58 ± 1.83
	+6	25	0.474 ± 0.179	0.123 ± 0.049	21.97 ± 29.08	6.74 ± 3.05	60.95 ± 44.88	307.85 ± 23.03	23.98 ± 3.44

Statistics

Table S2.2: Results of the Kruskal-Wallis tests for effects of temperature-amplitude (TA) on survival and the repeated measures two-way ANOVA for effects of TA on the increase of size from initial (increase %) of *Saccharina latissima* across latitudes in Europe. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects				
survival %	Population (P)	Kruskal-Wallis	4	9.265	0.055
	Temperature-Amplitude (TA)	Kruskal-Wallis	3	9.675	0.022* [x]
	Within-subjects effects				
survival % Spitsbergen	TA	*all survived*	/	/	/
survival % Bodø	TA	Kruskal-Wallis	3	3.000	0.392
survival % Bergen	TA	Kruskal-Wallis	3	3.000	0.392
survival % Helgoland	TA	Kruskal-Wallis	3	3.000	0.078
survival % Locmariaquer	TA	Kruskal-Wallis	3	10.2857	0.016* [x]
	two-way ANOVA				
increase %[a]	Population (P)		4	1473.251	< 0.001*
	Temperature-Amplitude (TA)		3	11.444	< 0.001*
	Day		4	149.348	< 0.001*
	P × TA		12	6.084	< 0.001*

[a] $\log_{10}$ transformation
[x] Dunn-Bonferroni's test: reject H_0 if $p \leq$ alpha/2, alpha = 0.05

Table S2.3 Results of the Friedman tests for effects of temperature-amplitude on the photosynthetic efficiency F_v/F_m of *Saccharina latissima* across latitudes in Europe measured on day 1 – 8. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	χ^2 value	*p* value
	Between-subjects effects			
F_v/F_m	Population (P) + Temperature-Amplitude (TA) + Day	19	102.86	< 0.001*
	Within-subjects effects			
F_v/F_m TA	P + Day	4	19.275	< 0.001*
F_v/F_m Population	TA + Day	3	16.470	< 0.001*

Table S2.4: Results of the two-way ANOVA, one-way ANOVA or Kruskal-Wallis test for effects of temperature-amplitude on chlorophyll a (Chl *a*), pool size of accessory pigments (Acc), pool size of all xanthophylls (VAZ = violaxanthin [V], antheraxanthin [A], zeaxanthin [Z]), de-epoxidation state of the xanthophyll cycle (DPS) and Chl*a*:Acc ratio in *Saccharina latissima* across latitudes in Europe. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	two-way ANOVA				
Chl *a*	Population (P)		4	26.616	< 0.001*
	Temperature-Amplitude (TA)		3	0.419	0.740
	P × TA		12	1.086	0.388
	two-way ANOVA				
Acc	Population (P)		4	40.995	< 0.001*
	Temperature-Amplitude (TA)		3	0.649	0.586
	P × TA		12	0.933	0.521
	Between-subjects effects				
VAZ	Population (P)	Kruskal-Wallis	4	26.192	< 0.001* x
	Temperature-Amplitude (TA)	Kruskal-Wallis	3	5.645	0.130
	Within-subjects effects				
VAZ Spitsbergen	TA	Kruskal-Wallis	3	1.897	0.594
VAZ Bodø	TA	Kruskal-Wallis	3	6.375	0.095
VAZ Bergen	TA	ANOVA	3	0.439	0.729
VAZ Helgoland	TA	Kruskal-Wallis	3	8.096	0.044* x
VAZ Locmariaquer	TA	ANOVA	3	1.933	0.178
	Between-subjects effects				
DPS	Population (P)	Kruskal-Wallis	4	12.579	0.014* x
	Temperature-Amplitude (TA)	Kruskal-Wallis	3	8.060	0.045* x
	Within-subjects effects				
DPS Spitsbergen	TA	ANOVA	3	0.602	0.602
DPS Bodø	TA	ANOVA	3	3.083	0.068
DPS Bergen	TA	ANOVA	3	1.616	0.237
DPS Helgoland	TA	Kruskal-Wallis	3	11.272	0.010* x
DPS Locmariaquer	TA	Kruskal-Wallis	3	12.265	0.007* x
	Between-subjects effects				
Chl*a*:Acc	Population (P)	Kruskal-Wallis	4	41.377	< 0.001* x
	Temperature-Amplitude (TA)	Kruskal-Wallis	3	1.928	0.587

continues on next page

	Within-subjects effects				
Chl*a*:Acc Spitsbergen	TA	ANOVA	3	0.555	0.654
Chl*a*:Acc Bodø	TA	ANOVA	3	0.797	0.519
Chl*a*:Acc Bergen	TA	ANOVA	3	1.410	0.288
Chl*a*:Acc Helgoland	TA	ANOVA	3	7.161	0.005*
Chl*a*:Acc Locmariaquer	TA	Kruskal-Wallis	3	3.154	0.368
	Between-subjects effects				
Chl*a*:VAZ	Population (P)	Kruskal-Wallis	4	46.347	< 0.001* [x]
	Temperature-Amplitude (TA)	Kruskal-Wallis	3	5.241	0.155
	Within-subjects effects				
Chl*a*:VAZ Spitsbergen	TA	ANOVA	3	1.534	0.256
Chl*a*:VAZ Bodø	TA	Kruskal-Wallis	3	3.199	0.362
Chl*a*:VAZ Bergen	TA	ANOVA	3	0.066	0.996
Chl*a*:VAZ Helgoland	TA	ANOVA	3	12.620	< 0.001*
Chl*a*:VAZ Locmariaquer	TA	ANOVA	3	11.430	< 0.001*

[x] Dunn-Bonferroni's test: reject H_0 if $p \leq$ alpha/2, alpha = 0.05

Table S2.5: Results of the two-way ANOVA for effects of temperature-amplitude on the C:N ratio, total C and total N content in *Saccharina latissima* across latitudes in Europe. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects				
C:N	Population (P)	Kruskal-Wallis	4	36.807	< 0.001*[x]
	Temperature-Amplitude (TA)	Kruskal-Wallis	3	0.294	0.961
	Within-subjects effects				
C:N Spitsbergen	TA	ANOVA	3	2.099	0.154
C:N Bodø	TA	ANOVA	3	0.150	0.927
C:N Bergen	TA	ANOVA	3	6.202	< 0.001*
C:N Helgoland	TA	Kruskal-Wallis	3	0.596	0.897
C:N Locmariaquer	TA	ANOVA	3	2.507	0.109
	two-way ANOVA				
Total C	Population (P)		4	9.835	< 0.001*
	Temperature-Amplitude (TA)		3	0.679	0.569
	P × TA		12	1.566	0.128
	Between-subjects effects				
Total N	Population (P)	Kruskal-Wallis	4	36.084	< 0.001*[x]
	Temperature-Amplitude (TA)	Kruskal-Wallis	3	1.180	0.758
	Within-subjects effects				
Total N Spitsbergen	TA	Kruskal-Wallis	3	2.382	0.497
Total N Bodø	TA	ANOVA	3	0.808	0.513
Total N Bergen	TA	ANOVA	3	3.863	0.041*
Total N Helgoland	TA	Kruskal-Wallis	3	1.191	0.755
Total N Locmariaquer	TA	ANOVA	3	2.538	0.106

[x] Dunn-Bonferroni's test: reject H_0 if $p \leq$ alpha/2, alpha = 0.05

Table S2.6: Results of the two-way ANOVA for effects of temperature-amplitude on mannitol in *Saccharina latissima* across latitudes in Europe. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*F* value	*p* value
	two-way ANOVA			
Mannitol	Population (P)	4	19.605	< 0.001*
	Temperature-Amplitude (TA)	3	1.138	0.341
	P × TA	12	0.624	0.813

Table S2.7: Results of the two-way ANOVA for effects of temperature-amplitude on phlorotannins in *Saccharina latissima* across latitudes in Europe. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*F* value	*p* value
	two-way ANOVA			
Phlorotannins	Population (P)	4	29.123	< 0.001*
	Temperature-Amplitude (TA)	3	0.298	0.827
	P × TA	12	0.279	0.991

3. Publication II:

Seasonal and inter-annual variability in the heatwave tolerance of the kelp *Saccharina latissima*

Sarina Niedzwiedz, Nora Diehl, Kai Bischof

Author to be included before submission: Philipp Fischer

in preparation

Title: Seasonal and inter-annual variability in the heatwave tolerance of the kelp *Saccharina latissima*

Authors: Sarina Niedzwiedz, Nora Diehl, Kai Bischof

Abstract

The geographical distribution of organisms, such as the foundation kelp species *Saccharina latissima*, is mainly driven by temperature. Globally increasing sea surface temperature (SST) and further intensification of marine heatwaves have already resulted in local extinction of kelp populations. In this study, we assessed temporal variation in the thermal susceptibility of *S. latissima*. Therefore, we compared stress responses of *S. latissima* field sporophytes sampled from Helgoland (German Bight) in June 2018, August 2018, and August 2019 to heatwave scenarios. We analyzed survival, growth, maximum quantum yield of photosystem II (F_v/F_m), and pigment composition. Survival decreased with increasing experimental and environmental temperatures. Growth revealed seasonal patterns, being higher in June than in August, while F_v/F_m decreased with increasing temperature, independently of the sampling time. We found an increase in the concentration of light harvesting pigments and in the de-epoxidation state of the xanthophyll cycle (DPS) with higher treatment temperature. These increases intensified with higher environmental temperature prior to the experiment (June 2018 < August 2019 < August 2018). Our results indicate overall high plasticity of *S. latissima* to temperature increase and enhanced susceptibility to heatwaves in a prevailing high-temperature environment, e.g. during years with higher mean SST.

Introduction

Temperature is considered one of the most limiting factors for the global distribution and abundance of species (Lüning 1990, Adey & Steneck 2001), as it directly affects metabolic rates and enzyme activities (Q_{10} rule) (Bannwarth et al. 2013). Response traits as survival, growth and reproduction integrate the temperature tolerance of a given species over a multitude of enzymatic reactions (Pörtner & Farrell 2008, Clarke & Fraser 2017). These traits vary with life-history stages (Martins et al. 2017) and are affected by the interaction with other biotic or abiotic factors (e.g. Feehan et al. 2012, Diehl et al. 2020). Close to the species-specific temperature tolerance limit, energy requirements generally increase (DeWitt et al. 1998, Pörtner et al. 2005), which is reflected in changes in physiological and biochemical response parameters. The ability of a species to modify its phenotype and thereby acclimate to different temperatures (phenotypic plasticity) (King et al. 2018), determines its temperature tolerance and, ultimately, latitudinal distribution. Broadly distributed seaweeds are reported to have a wider temperature tolerance (eurytherm) than species with limited distribution (Wiencke et al. 1994), however, certain species do not necessarily tolerate large local temperature variation (Kelly et al. 2012). In fact, an extensive seaweed loss due to high temperatures could already be shown for southern marginal populations (Wernberg et al. 2018, Filbee-Dexter et al. 2020).

Hitherto, the global temperature rise is one of the major threats to coastal ecosystems, resulting in a poleward migration and, eventually, local extinctions of species (Perry et al. 2005, Pörtner & Knust 2007). The global mean sea surface temperature (SST) has already risen by ~ 0.63 °C since 1850 and is expected to increase further (IPCC 2019). Additionally, extreme temperature events, such as marine heatwaves (MHWs), are predicted to intensify globally in the future (Hobday et al. 2016, Oliver et al. 2018). Severe effects of MHWs on structures of marine ecosystems have been recorded around the globe.

Even far North of their southernmost distributional edge species can reach their thermal tolerance limit in regions with great seasonal and inter-annual temperature variation. Bennett et al. (2015) reported that rear-edge and central populations of an Australian brown macroalga exhibited equal sensitivity to warming. SSTs around Helgoland (North Sea, German Bight) reach from −1 °C to +21 °C throughout the year, with an annual average of 10.1 °C (Boersma et al. 2016). Over the past 50 years, a warming trend of +0.037 °C per year of the annual mean SST was estimated, with both winters and summers becoming warmer (Wiltshire et al. 2010). The apparent changes in the marine ecosystem around Helgoland have

been attributed to the increasing SST, such as shifts in the macroalgal community and changes in depth distribution of kelp species (Bartsch & Kuhlenkamp 2000, Pehlke & Bartsch 2008).
Kelps (Phaeophyceae, Laminariales) are important foundation species acting as ecosystem engineers, providing habitat, food and nursery grounds for many associated organisms (Eckman et al. 1989, Schultze et al. 1990, Teagle et al. 2017). They support a wide range of ecosystem services, e.g. increased fisheries, coastline protection or carbon storage. The value of kelp forests is estimated to US$ ~ 0.5 – 1 million per year and kilometer of coastline (Wernberg et al. 2018). Broad-scale declines and loss of marine forests were reported along the coasts of Australia and the North Atlantic (Smale & Wernberg 2013, Filbee-Dexter & Wernberg 2018, Filbee-Dexter et al. 2020). More severe losses of kelp populations were observed after extraordinarily high SSTs in the North Atlantic, and it was proposed that kelp mortality increases in warmer years (Andersen et al. 2013, Filbee-Dexter et al. 2020). Contrary, the beneficial acclimation hypothesis (BAH) for organisms describes a performance advantage after acclimation to a particular environment (Leroi et al. 1994, Deere & Chown 2006) and, hence, would count for local acclimation of kelp populations to high-temperature conditions.
Saccharina latissima (Laminariales) is a boreal-temperate kelp species (Van den Hoek et al. 1993), forming underwater forests along the entire European Atlantic coast from Svalbard to Northern Portugal (Araújo et al. 2016). The species exhibits various mechanisms to adjust to environmental conditions (reviewed by Bartsch et al. 2008). For instance, pronounced physiological circannual rhythms have been described for *S. latissima*. On Helgoland, the main growth activity was measured in spring, peaking in July before it decreases substantially, though not completely ceasing, towards the end of summer (Lüning 1979). The optimum growth temperature of *S. latissima* sporophytes usually ranges between 10 and 15 °C (Lüning 1979, Fortes & Lüning 1980), and the lethal temperature was determined at 23 °C (Bolton & Lüning 1982). However, Diehl et al. (submitted) detected that the species can even survive temperatures up to 25 °C for more than a week after successive temperature increase, such as MHWs.
The aim of the present study was to investigate the significance of the seasonal thermal history for high-temperature responses of *S. latissima*. Therefore, we conducted heatwave-like experiments with field-collected sporophytes from Helgoland, applying temperatures reaching the upper temperature tolerance limit for the species (18 – 24 °C). The experiments were

run in August 2018 and August 2019 to investigate inter-annual differences. Additionally, seasonal effects were evaluated by comparing the data from June 2018 (Diehl et al. submitted) to the results from August 2018.

We hypothesized that physiological and biochemical responses of *S. latissima* to heat stress are affected by the local temperature history and, thus, varies between seasons and between years. Based on the major variation of the SST in the inter-annual comparison between August 2018 and August 2019, we expect *S. latissima* to show a reduced temperature tolerance to heatwaves in the year with overall higher local temperature. Comparing seasons, we expect higher thermal susceptibility of *S. latissima* in August 2018 than in June 2018.

Material and methods

Sea surface temperature (SST)

The SST around Helgoland is monitored continuously by the COSYNA (Coastal Observation System for Northern and Arctic Seas) underwater node system (Fischer et al. 2020) in the MarGate experimental field in a depth of 10 m, providing real-time measurements of the SST. The data is freely available and can be downloaded at: https://dashboard.awi.de/?dashboard=10421. The daily optimum interpolation of sea surface temperature (daily OISST) was provided by Reynolds et al. (2007) and Banzon et al. (2020). The data was displayed in the context of marine heatwaves and modified after Schlegel (2020).

Sampling August 2018/2019

Saccharina latissima sporophytes were collected by scientific SCUBA divers around Helgoland from a high tide depth of 3 – 5 m. Samplings were conducted on the 23rd of August 2018 and 21st of August 2019 (**Fig. 3.1a**) (N 54°11.3', E 7°54.2').

Experimental set-up August 2018/2019

The experimental set-up was based on the temperature-amplitude experiment by Diehl et al. (submitted). Meristematic discs (Ø 2.2 – 2.4 cm) were cut and afterwards equally distributed between treatments and replicates (n = 4), avoiding pseudo-replicates, and cultivated in aerated 2-L clear-plastic bottles, filled with sterile ½ Provasoli enriched seawater (½ PES, Provasoli 1968 with the following modifications: HEPES-buffer instead of TRIS, double concentration of Na_2glycerophosphat, iodine enrichment after Tatewaki 1966; S_A 33), applying a 16/8-light/dark cycle of 30 – 35 µmol photons m^{-2} s^{-1} (ProfiLux 3 with LED Mitras daylight 150, GHL Advanced Technology, Kaiserslautern, Germany). Treatment temperatures (18, 20, 22, 24 °C) were chosen after Diehl et al. (submitted), based on 18 °C summer mean temperature on Helgoland as control, and Δ+2, Δ+4, Δ+6 °C as temperature-amplitude treatments, mimicking marine heatwaves. The experiments ran for 14 days (**Fig. 3.1b**), starting with an acclimation phase (t_0 – t_7) in which the temperature of the treatments was increased by 2 °C every second day until the respective treatment temperature was reached. Temperature treatments ran for 7 days (t_8 – t_{14}).

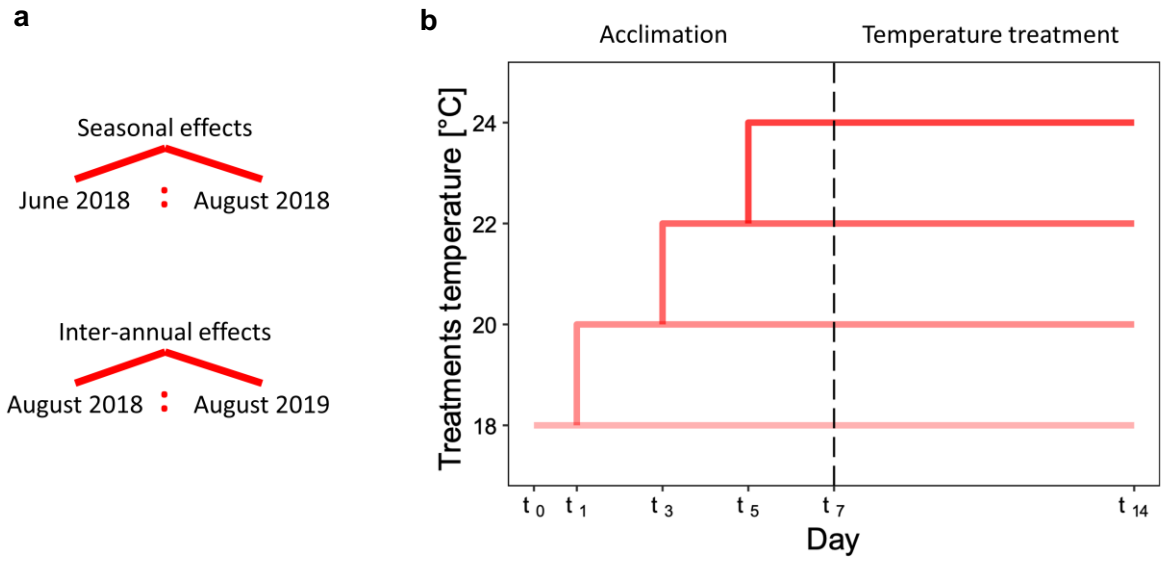


Fig. 3.1: Experimental set-up. **a:** Seasonal vs. inter-annual effects: Seasonal effects in the short-term high-temperature response of *Saccharina latissima* were assessed by comparing the samplings of June 2018 to August 2018. Those were compared to the inter-annual effects studied by the samplings of August 2018 to August 2019. For all comparisons of the short-term temperature response of *S. latissima* two weeks of experimental temperature treatment were applied. **b:** Experimental temperature treatment: Dotted line separates the acclimation phase (t_0 – t_7) and the experimental temperature treatment (t_8 – t_{14}; 18, 20, 22, 24 °C). During acclimation, the temperature was increased by 2 °C every second day (t_1, t_3, t_5) until the treatment's final temperature was reached (n = 4 for each temperature treatment). Physiological parameters and pigments were measured on t_0 and t_{14}.

Data-set June 2018

Sporophytes were collected on the 26th of June 2018 (N 54°10.9', E 7°55.1'). The experiment was conducted and data collected by Diehl et al. (submitted). Raw data were downloaded from the PANGAEA data platform and re-evaluated for this study. Initial pigment values (t_0) were not measured in June 2018 and are therefore missing.

Physiological response variables

Survival. Survival between t_0 and t_{14} was calculated as percentage for each replicate of the temperature treatments (**Equation 1**). As most of the meristematic discs started to disintegrate throughout the experiment, discs were only considered 'dead', if they were completely decomposed or if no maximum quantum yield of photosystem II (F_v/F_m) was detectable anymore.

$$Survival\ [\%] = \frac{\sum number\ of\ discs\ t_{14}}{\sum number\ of\ discs\ t_0} \cdot 100 \qquad \textbf{Equation 1}$$

Size. Digital photographs of the discs were taken on t_0 and t_{14} to analyze the change in size (cm^2) (growth and decomposition) during the experiment. The pictures were analyzed with ImageJ 1.53a (java 1.8.0_172 [64-bit]). Mean size of all surviving discs per replicate was determined on both t_0 and t_{14} by summing up the discs' size for each replicate and dividing it by the number of remaining discs. Changes in size during the experiment was shown as percentage for each replicate of the temperature treatments (**Equation 2**).

$$Area\ [\%] = \frac{\sum area\ of\ discs\ t_{14} \Big/ \sum number\ of\ discs\ t_{14}}{\sum area\ of\ discs\ t_0 \Big/ \sum number\ of\ discs\ t_0} \cdot 100 \qquad \textbf{Equation 2}$$

Maximum quantum yield of photosystem II (F_v/F_m). F_v/F_m was measured on t_0 and t_{14} using pulse amplitude modulated fluorometers (June 2018/August 2018: ImagingPAM, Walz Imaging PAM Maxi Version M-series; August 2019: Portable Chlorophyll Fluorometer PAM-2100, Heinz Walz GmbH, Effeltrich, Germany). Prior to the measurements, the discs were dark adapted for 5 min. Changes in F_v/F_m between t_0 and t_{14} was shown as percentage for each replicate of the temperature treatments (**Equation 3**).

$$F_v/F_m\ [\%] = \frac{F_v/F_m\ t_{14}}{F_v/F_m\ t_0} \cdot 100 \qquad \textbf{Equation 3}$$

Pigment composition

Pigment composition was analyzed according to Koch et al. (2015). For each sample, 50 – 100 mg of the freeze-dried, powdered material was extracted in 1 mL 90 % acetone (volume percent, v/v) at 4 °C for 24 h in darkness. The supernatant was filtered and then analyzed by an High Performance Liquid Chromatography (HPLC; LaChromElite® system, L-2200 autosampler [chilled], DA-detector L-2450; VWR-Hitachi International GmbH, Darmstadt, Germany), applying a gradient according to Wright et al. (1991) and separating the pigments by a Spherisorb® ODS-2 column (250 × 4.6 mm, 5 µm; Waters, Milford, MA, USA). Modification was as follows: The mobile phase was 0.5 mmol sulfuric acid (H_2SO_4), run at a flow rate of 0.4 mL min^{-1}, 5 – 40 bar, 75 °C. To identify and quantify pigment peaks, the respective standard for each pigment was used (DHI Lab Products, Hørsholm, Denmark).

The accessory pigment (Acc) and xanthophyll cycle pigment (VAZ) concentration (mg g^{-1} dry weight [DW]) were calculated by the sum of chlorophyll *c*2 and fucoxanthin and the sum of violaxanthin, zeaxanthin and antheraxanthin concentration, respectively (**Equation 4**, **Equation 5**).

$$Acc\ [mg\ g^{-1}\ DW] = Chlorophyll\ c2 + Fucoxanthin \qquad \textbf{Equation 4}$$

$$VAZ\ [mg\ g^{-1}\ DW] = Violaxanthin + Zeaxanthin + Antheraxanthin \qquad \textbf{Equation 5}$$

The ratios of Acc and VAZ to chlorophyll *a* (Chl *a*) were determined, respectively (**Equation 6**, **Equation 7**).

$$Acc{:}\,Chla = \frac{[Chlorophyll\ \mathrm{c2} + Fucoxanthin]}{[Chlorophyll\ \mathrm{a}]} \qquad \textbf{Equation 6}$$

$$VAZ{:}\,Chla = \frac{[Violaxanthin + Antheraxanthin + Zeaxanthin]}{[Chlorophyll\ \mathrm{a}]} \qquad \textbf{Equation 7}$$

The de-epoxidation state of the xanthophyll cycle (DPS) was determined after Colombo-Pallotta et al. (2006) (**Equation 8**):

$$DPS = \frac{Zeaxanthin + 0.5 \cdot Antheraxanthin}{Violaxanthin + Zeaxanthin + Antherxanthin} \qquad \textbf{Equation 8}$$

Statistical analysis

To test for the influence of the thermal history, seasonal (June 2018 vs. August 2018) and inter-annual (August 2018 vs. August 2019) effects were investigated separately. Significant differences of the response variables to the interactive fixed-effects temperature (T) × sampling time (ST) were assessed using a fitted linear model using analyses of variances (two-way ANOVA) and post hoc Tukey's tests ($p < 0.05$). Normality of residuals (Shapiro-Wilk test, $p > 0.05$) and homoscedasticity (Levene's test, $p > 0.05$) were tested. As *F*-statistic was reported to be robust against a moderate violation of normal distributions at small sample sizes in terms of Type I errors and no beneficial effects of transformation were found (Blanca et al. 2017), the non-parametric data-sets were not transformed. All statistical analyses of the experiment were run in RStudio (Version 1.3.1093). Statistical results are summarized in the supplements (**Tables S3.1 – S3.3**).

Results

Seasonal and inter-annual temperature variation

The course of the sea surface temperature (SST) around Helgoland between April and September 2018 and 2019 is compared in **Figure 3.2a**. While the monthly mean SST (data not shown) was up to 3 °C lower in April, May and June 2018, it was max. 2 °C higher in July and August 2018 than in the respective month 2019 (**Fig. 3.2b**).

Three marine heatwaves (MHWs) occurred within the investigated period in 2018, lasting 37 days in total. The highest temperatures were recorded at the end of July 2018, is classified as category II MHW 'strong', being the 2 – 3-fold of the difference of 90th percentile from the mean climatology value (Hobday et al. 2016, 2018, Schlegel 2020) (**Fig. 3.2c**). Contrary, only one MHW was observed between April and September in 2019, starting after the sampling campaign at the end of August (Schlegel 2020) (**Fig. 3.2d**).

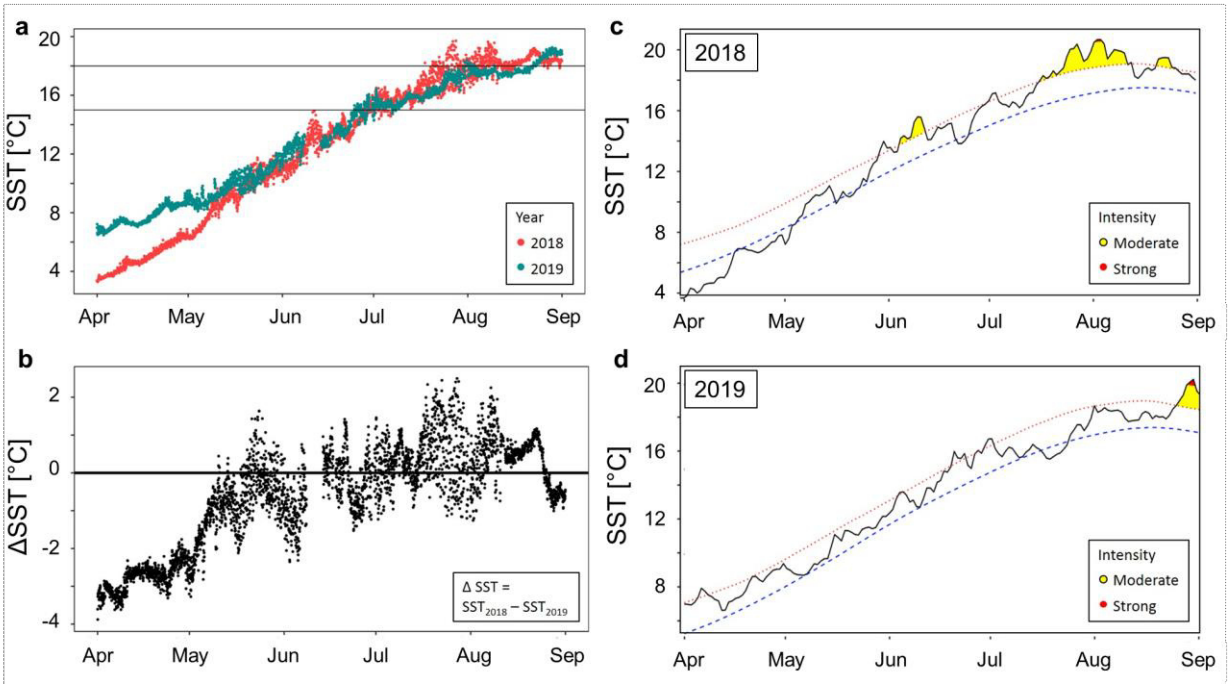


Fig. 3.2: Sea surface temperature (SST) around Helgoland. **a:** Hourly mean SST (°C) in 2018 (red) and 2019 (turquoise) from April to August. 15 °C marks the upper optimum temperature of *Saccharina latissima* (Fortes & Lüning 1980). 18 °C marks the control temperature of the experiment after Diehl et al. (submitted). **b:** Difference between corresponding SST measurements at each time point 2018 and 2019 (ΔSST) (°C). 0 = SST_{2018} = SST_{2019}. **c & d:** Black lines show the satellite-derived SST in 2018 and 2019 on Helgoland. Red dashed lines represent the 90th percentile of the average SST over 30 years (blue dashed line) after Hobday et al. (2016). Categories after Hobday et al. (2018): Moderate: 1 – 2-fold difference of 90th percentile from the mean climatology value. Strong: 2 – 3-fold difference of 90th percentile from the mean climatology value. Data provided by Reynolds et al. (2007) and Banzon et al. (2020); modified after Schlegel (2020).

Physiology and pigment composition

Seasonal effects – June 2018 vs. August 2018

Survival. Survival of discs was not affected by interactive effects of temperature (T) and sampling time (ST) (T×ST) (**Fig. 3.3a**, **Supplement Table S3.1**). However, T had an impact in August 2018 ($p < 0.001$) when survival was 75 % lower in the 24 °C than in the 18 °C treatment ($p < 0.01$). In June 2018, a decreasing trend in survival with increasing treatment temperature was observed. Furthermore, the ST had an impact ($p < 0.001$) with 40 – 79 % more discs surviving in June 2018 compared to August 2018 in each temperature treatment ($p < 0.01$).

Size. Size was neither affected by T alone nor by T×ST interaction (**Fig. 3.3b**, **Table S3.1**), whereas ST was detected to affect the discs' size ($p < 0.001$). Compared to the initial size (t_0), discs grew between 16 – 20 % in all temperature treatments in June 2018, while the discs' size decreased in August 2018 during the experiment. Regarding the 24 °C treatment, the discs in August 2018 were 45 % smaller than in June 2018 ($p < 0.01$).

Maximum quantum yield of photosystem II (F_v/F_m). F_v/F_m was only affected by T ($p < 0.001$) and not by ST or T×ST interaction. (**Fig. 3.3c**, **Table S3.1**). Independently from the season, F_v/F_m decreased at enhanced temperatures (June 2018: 24 °C < [22 °C = 20 °C = 18 °C]; August 2018: [24 °C = 22 °C] < [20 °C = 18 °C]; $p < 0.01$).

Chl a *& Acc.* The interaction of temperature (T) and sampling time (ST) affected the chlorophyll *a* (Chl *a*) and accessory pigment (Acc) concentration (**Table 3.1**, **Table S3.2**, $p < 0.05$), while T alone had no impact. Nevertheless, Chl *a* and Acc increased with higher temperature in August 2018 ($p < 0.05$). No temperature-induced responses were observed in June 2018. The main effect of ST only had an impact on Acc ($p < 0.05$), being higher in August 2018 than in June 2018.

*Acc:Chl*a. We detected significant seasonal impacts of T and ST as main effects, but also a significant T×ST interaction for the Acc:Chl*a* ratio (**Fig. 3.4a**, **Table S3.3**, $p < 0.05$). In June 2018, Acc:Chl*a* showed no temperature-induced responses, while in August 2018, it decreased by 48 – 58 % comparing the 18 °C to the 22 °C and 24 °C treatment ($p < 0.05$).

*VAZ:Chl*a. The ratio of the xanthophyll pool size to chlorophyll *a* (VAZ:Chl*a*) was affected by the T×ST interaction (**Fig. 3.4b**, **Table S3.3**, $p < 0.01$). Furthermore, T affected VAZ:Chl*a* ($p < 0.001$). Although, no temperature-induced significances were detected in August 2018, in June 2018 VAZ:Chl*a* was 47 – 54 % higher in the 24 °C treatment than in the 18 °C, 20 °C and 22 °C treatments ($p < 0.001$). The effect of ST in the different seasons ($p < 0.001$) became apparent comparing the 24 °C treatments. In June 2018 VAZ:Chl*a* was 58 % higher than in the 24 °C treatment in August 2018 ($p < 0.001$).

DPS. The de-epoxidation state of the xanthophyll cycle (DPS) was affected by the interaction of T and ST, resulting in differences between temperatures at different sampling times (**Fig. 3.4c**, **Table S3.3**, $p < 0.05$). DPS increased with higher treatment temperatures (June 2018: 24 °C < [22 °C = 20 °C = 18 °C], $p < 0.001$; August 2018: [24 °C = 22 °C] < [22 °C = 20 °C = 18 °C], $p < 0.05$). Furthermore, ST had an impact on DPS comparing June and August 2018 ($p < 0.001$), being higher in August than in June.

Inter-annual effects – August 2018 vs. August 2019

Survival. T affected the survival of discs ($p < 0.001$), however, the interaction of T and ST did not (**Fig. 3.3a**, **Table S3.1**). In both years, survival was 64 – 75 % higher in the 18 °C than in the 24 °C treatment, being significant in August 2019 (24 °C < [22 °C = 20 °C = 18 °C]; $p < 0.05$). In total, more discs survived the treatments in August 2019 (64 %) than in August 2018 (38 %).

Size. Neither T nor T×ST affected the size (**Fig. 3.3b**, **Table S3.1**). Only ST had an impact ($p < 0.001$), with the discs being smaller in August 2019 than in August 2018.

Maximum quantum yield of photosystem II (F_v/F_m). F_v/F_m was neither affected by interactive effects of T and ST nor by ST (**Fig. 3.3c**, **Table S3.1**, $p > 0.05$). Nevertheless, T affected F_v/F_m ($p < 0.001$), resulting in lower values with increasing treatments temperature (August 2018: [24 °C = 22 °C] < [18 °C = 20 °C], August 2019: 24 °C < [18 °C = 20 °C = 22 °C]; $p < 0.01$).

Chl a *& Acc.* Chl *a* and Acc were neither affected by interactive effects of T and ST, nor by ST alone (**Table 3.1**, **Table S3.2**, $p > 0.05$). T had an impact ($p < 0.01$). The Chl *a* and Acc concentrations were 33 – 84 % higher in the 24 °C than in the 18 °C treatment.

*Acc:Chl*a. Acc:Chl*a* differed significantly between the temperatures at different samplings (T×ST; $p < 0.001$) (**Fig. 3.4a**, **Table S3.3**). T alone also had an impact ($p < 0.01$), resulting in a

decrease of 48 – 58 %, comparing the 18 °C with the 22 °C and 24 °C treatment in August 2018 ($p < 0.05$). In August 2019, Acc:Chl*a* decreased by 47 – 63 % between t_0 and t_{14} ($p < 0.05$). Even though the general effect of ST was not significant, Acc:Chl*a* on t_0 was 53 % higher in August 2019 than in August 2018.

*VAZ:Chl*a. VAZ:Chl*a* was not affected by T×ST interaction (**Fig. 3.4b**, **Table S3.3**). T affected VAZ:Chl*a* ($p < 0.001$), resulting in 54 % lower ratios in the 18 °C than in the 22 °C and 24 °C temperature treatment in August 2018 ($p < 0.05$). In August 2019, no temperature-induced significances were detected. Furthermore, the ST also had an effect on VAZ:Chl*a* ($p < 0.001$), resulting in higher values in August 2018 than in August 2019.

DPS. DPS was not affected by T×ST, or ST (**Fig. 3.4c**, **Table S3**). Nevertheless, T alone had a strong impact on DPS ($p < 0.001$). DPS was considerably higher at 24 °C (August 2018 and 2019: [24 °C = 22 °C] < [22 °C = 20 °C = 18 °C], $p < 0.05$).

Table 3.1: Chlorophyll *a* (Chl *a*) and accessory pigment (Acc) concentration (µg g^{-1} dry weight) of *Saccharina latissima* at the beginning of the experiment (t_0) and after two weeks of acclimation and temperature treatments (t_{14}) (18, 20, 22, 24 °C) in June 2018, August 2018 and August 2019. t_0 is missing for June 2018. Different lowercase letters indicate significant differences between treatments in June and August 2018 ($p < 0.05$). Different capital letters indicate significant differences between treatments in August 2018 and August 2019 ($p < 0.05$).

	Temper-	**June 2018**			**August 2018**				**August 2019**		
	ature	Mean	± SD	Sig.	Sig.	Mean	± SD	Sig.	Sig.	Mean	± SD
Chl *a*	18 °C – t_0	---	---	---	ab	978.8	744.1	AB	A	249.2	63.3
	18 °C – t_{14}	1254.1	213.8	ab	a	219.9	56.7	A	AB	584.5	129.1
	20 °C – t_{14}	1211.7	175.6	ab	b	1470.2	809.8	B	AB	844.1	326.3
	22 °C – t_{14}	1247.1	80.9	ab	ab	1023.5	572.0	AB	AB	742.6	276.2
	24 °C – t_{14}	959.8	257.0	ab	b	1346.0	231.2	B	AB	249.2	63.3
Acc	18 °C – t_0	---	---	---	ab	653.3	325.9	AB	AB	391.7	100.4
	18 °C – t_{14}	920.0	186.0	b	a	319.9	95.4	A	AB	319.9	95.4
	20 °C – t_{14}	802.4	230.4	b	b	920.0	186.0	B	AB	480.1	78.7
	22 °C – t_{14}	847.8	122.5	b	ab	802.4	230.4	AB	AB	802.4	230.4
	24 °C – t_{14}	652.1	273.2	ab	b	847.77	122.5	B	AB	623.0	158.9

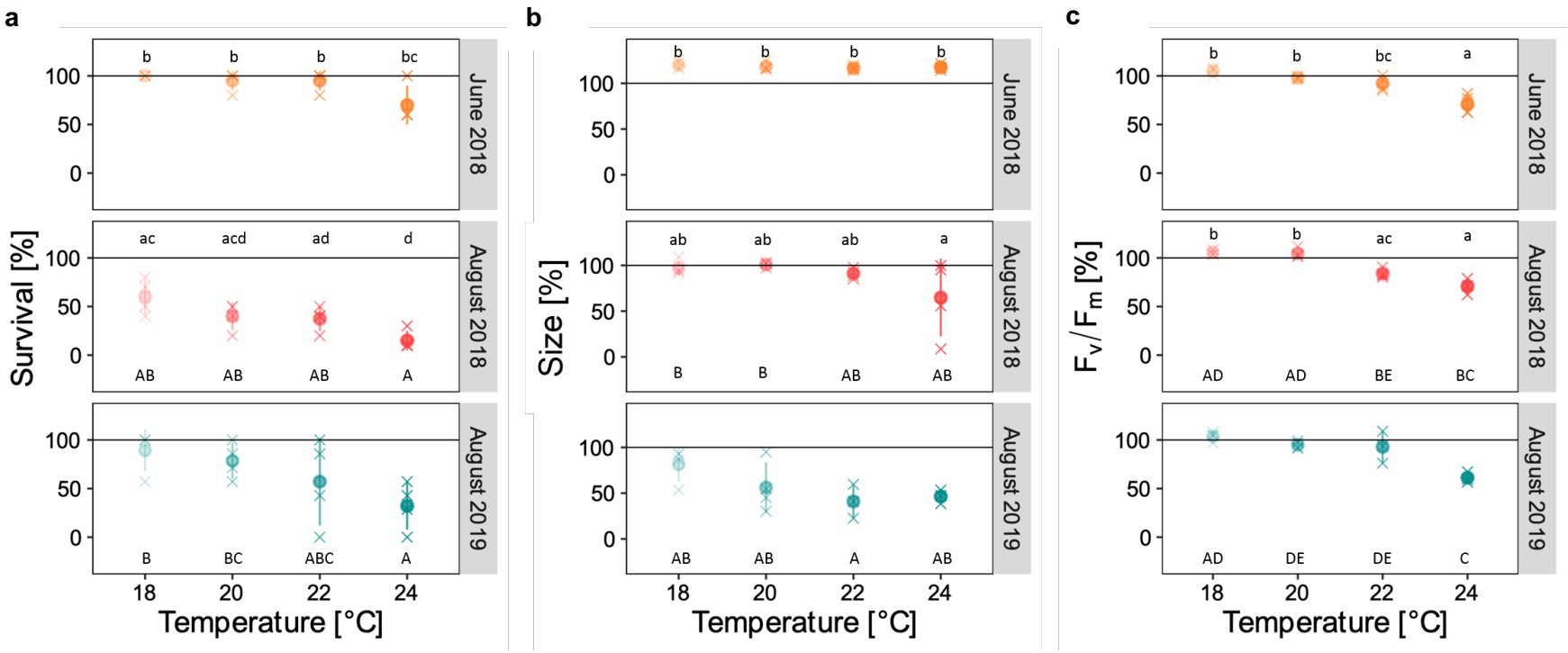


Fig. 3.3: Physiological responses (% of t_0) of *Saccharina latissima* after two weeks of acclimation and temperature treatments (18, 20, 22, 24 °C) separated into panels by sampling time (June 2018, August 2018, August 2019). Treatment mean (dots) ± SD (n = 3 – 4) and average replicate response (cross). **a:** Survival. **b:** Size. **c:** Maximum quantum yield of photosystem II (F_v/F_m). Different lowercase letters indicate differences between treatments in June and August 2018 ($p < 0.05$). Different capital letters indicate significant differences between treatments in August 2018 and August 2019 ($p < 0.05$).

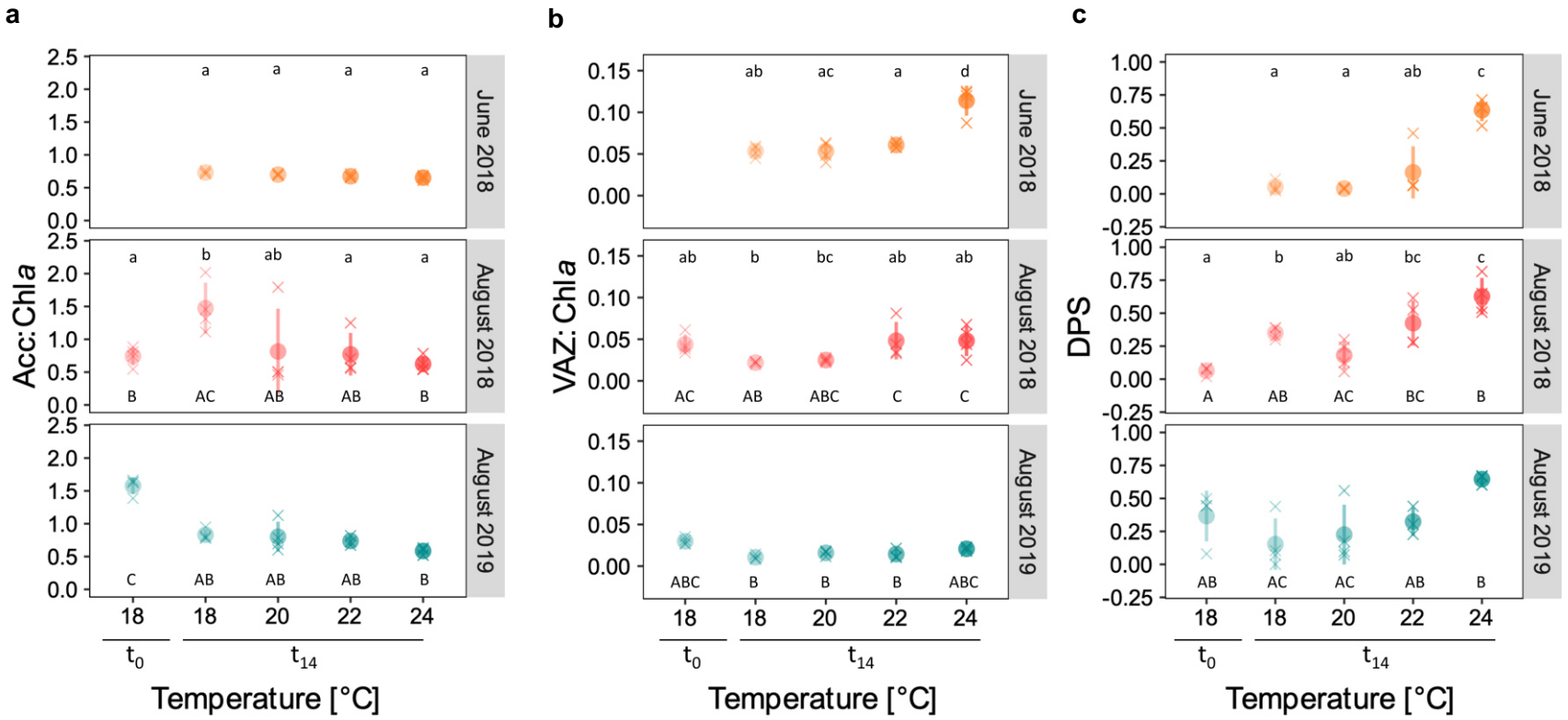


Fig. 3.4: Pigment ratios of *Saccharina latissima* at the beginning of the experiment (t_0) and after two weeks of acclimation and temperature treatments (t_{14}; 18, 20, 22, 24 °C) separated into panels by sampling time (June 2018, August 2018, August 2019). Treatment mean (dots) ± SD (n = 3 – 4) and average replicate response (cross). **a:** Accessory pigments to chlorophyll *a* (Acc:Chl*a*). **b:** Xanthophyll pool to chlorophyll *a* (VAZ:Chl*a*). **c:** De-epoxidation state of xanthophyll cycle (DPS). Different lowercase letters indicate significant differences between treatments in June and August 2018 ($p < 0.05$). Different capital letters indicate significant differences between treatments in August 2018 and August 2019 ($p < 0.05$).

Discussion

Recently, traits of *S. latissima* to MHWs in summer were shown to vary between populations across latitudes (Diehl et al. submitted). In their publication, Diehl and colleagues hypothesized that the environmental frame and seasonal temperature history might be a crucial factor regarding temperature tolerance of *S. latissima*. In this study, we assessed the influence of the thermal history on physiological and biochemical high-temperature responses of *Saccharina latissima*. We demonstrated that susceptibility to heatwaves largely varies between seasons and years. The observed differences were related to variation in sea surface temperature (SST) at the study site (Helgoland) between April and September 2018 and 2019. As individuals were exposed to different thermal conditions over longer periods throughout the year (thermal history) they constantly adjusted to the prevailing conditions to maintain performance.

Impact of the thermal history on kelp physiology

Independently from sampling time, *S. latissima* generally exhibited the highest mortality at 24 °C as also reported by Bolton & Lüning (1982). However, up to 70 % of the samples survived the heatwave treatment for more than a week, exceeding the prior reported upper survival limit of 23 °C (Bolton & Lüning 1982, Lüning 1984), potentially due to the stepwise temperature increase as suggested by Diehl et al. (submitted). Successive acclimation most probably supported the survival of extreme temperature conditions (Terblanche et al. 2007) and, thus, also decreases the susceptibility of *S. latissima* to natural heatwaves. Nevertheless, our results revealed a clear order in survival across sampling times (June 2018 > August 2019 > August 2018). Sporophytes were less tolerant and disintegrating at lower temperatures after longer periods of high mean SSTs or more MHW days prior to the experiment (June 2018 and August 2019 vs. August 2018). These results are consistent with observations in the field. Under high temperature stress, kelps were observed to start decomposing from the tip (Franke 2019, I. Bartsch pers. comm.). Less and shorter individuals were found at the sampling locations in August 2018 compared to June 2018 and August 2019 (S. Niedzwiedz pers. obs.).

Maximum quantum yield of photosystem II (PS II) (F_v/F_m) can be used as a proxy for the cellular stress level and algal vitality (Dring et al. 1996, Murchie & Lawson 2013, Hurd et al. 2014). In accordance with earlier studies, strong decreases of F_v/F_m were detected at higher temperatures in *S. latissima* (Fredersdorf et al. 2009, Hargrave et al. 2017, Diehl et al. 2020).

Contrary to the survival of the individuals, the decrease in F_v/F_m in this study was completely independent of the sampling time (season and year) and only responded to the absolute temperature. F_v/F_m is an immediate stress response variable (Dring et al. 1996), however, is less suited for long-term assessments, such as a significant influence of the thermal history (Murchie & Lawson 2013). Still, the results clearly state the negative influence of temperature extremes on the *in vivo* fluorescence, suggesting that the photosynthetic apparatus and the electron transfer of PS II have been impaired over time (Li et al. 2017). This might eventually result in cellular damage, e.g. due to enhanced generation of reactive oxygen species (ROS) (Foyer 2018).

The growth of *S. latissima* in this study followed the pattern observed by Lüning (1979), exhibiting season- and temperature-dependent activity. Fronds increase growth activity in spring, peaking in July and decreasing later in the year. Above and below the optimum temperature (10 – 15 °C), growth is reduced (Lüning 1979, Fortes & Lüning 1980, Bolton & Lüning 1982). Despite the fact of higher growth activity, we observed no temperature dependency since all remaining discs increased in size in June 2018. In contrast, in August 2018 and 2019, we determined a trend in decreasing discs' size with increasing temperature. Thus, high mortality was caused by massive tissue damage, as already described by Simonson et al. (2015).

Summarizing, long-term exposure to high temperatures prior to the experiment was not beneficial in terms of overall performance (beneficial acclimation hypothesis; BAH) (Leroi et al. 1994, Deere & Chown 2006) of *S. latissima*, and instead, thermal susceptibility to heatwaves decreases. These findings imply that the impact of MHWs varies between seasons, resulting in stronger reductions of marine forests at the end of summer and in overall warmer years.

High temperatures lead to increases in light harvesting complex pigments and DPS

Temperature stress is also reflected in the pigment composition of kelps (Andersen et al. 2013, Fernandes et al. 2016). Even though, we detected no changes in the pigment ratio (Acc:Chl*a*) of the light harvesting complex, absolute Chl *a* and Acc concentrations increased in August 2018 and 2019 with higher temperatures, which was in contrast to reports by Wernberg et al. (2016) and Nepper-Davidsen et al. (2019) who found a decrease in Chl *a* concentrations at suboptimal temperatures. In our experiment, an impact of light climate on pigment composition can be excluded since constant light conditions were maintained in all treatments.

Xanthophyll pigments (VAZ) are important part of non-enzymatic protective mechanisms (Demmig-Adams & Adams 1996). The reduction of violaxanthin to antheraxanthin to zeaxanthin (de-epoxidation state; DPS) (Demmig-Adams & Adams 1996) has mainly been described as a response to excessive light energy (e.g. Bischof et al. 2006), it also supports the intracellular heat dissipation and increases at enhanced temperatures (Nepper-Davidsen et al. 2019, Diehl et al. 2020, Li et al. 2020, Liesner et al. 2020a). However, recent studies demonstrated that temperature-induced stress is also compensated by an increase of DPS (Nepper-Davidsen et al. 2019, Diehl et al. 2020, Li et al. 2020, Liesner et al. 2020a) and was also confirmed in our study. Furthermore, we found DPS correlating with the thermal history of the sporophytes, being higher in August than in June. This might be due to an overall higher concentration of ROS level at the end of summer, supported by a variety of environmental factors, e.g. longer periods of warm temperature, higher light intensities and increased biotic strain, such as infections (Torres & Dangl 2005).

Ecological implications

The present study provides clear evidence that sampling time significantly affects the thermal susceptibility of *S. latissima* to heatwaves in summer. Comparing the observed seasonal and inter-annual differences, the decline in the performance of *S. latissima* was more pronounced at seasonal than at inter-annual comparisons. We observed an overall increased temperature tolerance in June than in August. Regarding inter-annual differences, we revealed that samples had a diminished temperature tolerance after exposition to a high-temperature environment. Hence, we confirm that declines and extinction of kelp populations will happen more likely in warmer years (Andersen et al. 2013, Filbee-Dexter et al. 2020) due to an overall high temperature strain on *S. latissima*. Thus, with the ongoing increase of the mean SST and intensification of MHWs (Oliver et al. 2018, IPCC 2019), extensive losses of *S. latissima* populations will not only occur at their rear-edge distribution range in the future. Still, successive temperature increase enables the species to maintain its integrative cell functions until up to 24 °C for shorter periods, and *S. latissima* generally reveals a high capacity to tolerate MHWs. However, our results indicate stronger effects on the *S. latissima* population in August, hence, mortality mainly increases during MHWs in late summer. The notion of large temporal variability in thermal response has to be considered in predictions of future range shifts in kelps based on experimental data from single experiments. Liesner et al. (2020b) highlighted the importance of cold seasons for kelp species, showing that gametogenesis and recruitment of

Laminaria digitata at high temperatures resulted in a decrease in the plasticity of the respective juvenile sporophytes to high temperatures. MHWs during the cold seasons might, therefore, not lead to an immediate loss of populations but further decrease the high temperature tolerance of kelp in summer months. Summarizing, Liesner et al. (2020b) and our study clearly demonstrate that the environmental frame and seasonal temperature history have a great influence on the performance and susceptibility of kelp species. These findings should be considered when interpreting previous studies and future studies on field sporophytes. Considering that not only temperature but also other environmental factors, e.g. light intensity and photoperiod, salinity or shifting biotic equilibria, affect the development of kelp in response to global warming, future re-distribution and the consequences in structure and functioning of the respective ecosystems might be even more severe than previously assumed.

Acknowledgements

The authors thank Dr. Inka Bartsch, Andreas Wagner (Alfred Wegener Institute for Polar and Marine Research), Britta Meyer-Schlosser (University of Bremen), and Dr. Eva-Maria Brodte and Uwe Nettelmann (Biologische Anstalt Helgoland) for providing facilities and technical support to conduct this study. Furthermore, we would like to thank the AWI diving team for sampling support on Helgoland.

References

Adey WH, Steneck RS (2001) Thermogeography over time creates biogeographic regions: A temperature/space/time-integrated model and an abundance-weighted test for benthic marine algae. *J Phycol* 37:677–698.

Andersen G, Pedersen M, Nielsen S (2013) Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima*. *J Phycol* 700:689–700.

Araújo RM, Assis J, Aguillar R, Airoldi L, Bárbara I, Bartsch I, Bekkby T, Christie H, Davoult D, Derrien-Courtel S, Fernandez C, Fredriksen S, Gevaert F, Gundersen H, Le Gal A, Lévêque L, Mieszkowska N, Norderhaug KM, Oliveira P, Puente A, Rico JM, Rinde E, Schubert H, Strain EM, Valero M, Viard F, Sousa-Pinto I (2016) Status, trends and drivers of kelp forests in Europe: an expert assessment. *Biodivers Conserv* 25:1319–1348.

Bannwarth H, Kremer BP, Schulz A (2013) Basiswissen Physik, Chemie und Biochemie, Third Edi. Springer Spektrum, Berlin Heidelberg.

Banzon V, Smith TM, Steele M, Huang B, Zhang HM (2020) Improved estimation of proxy sea surface temperature in the Arctic. *J Atmos Ocean Technol* 37:341–349.

Bartsch I, Kuhlenkamp R (2000) The marine macroalgae of Helgoland (North Sea): An annotated list of records between 1845 and 1999. *Helgol Mar Res* 54:160–189.

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J (2008) The genus *Laminaria sensu lato*: Recent insights and developments. *Eur J Phycol* 43:1–86.

Bennett S, Wernberg T, Joy BA, De Bettignies T, Campbell AH (2015) Central and rear-edge populations can be equally vulnerable to warming. *Nat Commun* 6:10280.

Bischof K, Rautenberger R, Brey L, Pérez-lloréns JL (2006) Physiological acclimation to gradients of solar irradiance within mats of the filamentous green macroalga *Chaetomorpha linum* from southern Spain. *Mar Ecol Prog Ser* 306:165–175.

Blanca MJ, Alarcón R, Arnau J, Bono R, Bendayan R (2017) Non-normal data: Is ANOVA still a valid option? *Psicothema* 29:552–557.

Boersma M, Grüner N, Tasso Signorelli N, Gonzalez PEM, Peck MA, Wiltshire KH (2016) Projecting effects of climate change on marine systems: is the mean all that matters? *R Soc Publ* 283:1–8.

Bolton JJ, Lüning K (1982) Optimal growth and maximal survival temperatures of Atlantic *Laminaria species* (Phaeophyta) in culture. *Mar Biol* 66:89–94.

Clarke A, Fraser KPP (2017) Why does metabolism scale with temperature? *Br Ecol Soc* 18:243–251.

Colombo-Pallotta MF, García-Mendoza E, Ladah LB (2006) Photosynthetic performance, light absorption, and pigment composition of *Macrocystis pyrifera* (Laminariales, Phaeophyceae) blades from different depths. *J Phycol* 42:1225–1234.

Deere JA, Chown SL (2006) Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. *Am Nat* 168:630–644.

Demmig-Adams B, Adams WW (1996) The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends Plant Sci* 1:21–26.

DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. *Tree* 13:77–81.

Diehl N, Karsten U, Bischof K (2020) Impacts of combined temperature and salinity stress on the endemic Arctic brown seaweed *Laminaria solidungula* J. Agardh. *Polar Biol* 43:647–656.

Diehl N, Roleda MY, Bartsch I, Karsten U, Bischof K (submitted) Summer heatwave impacts on the European kelp *Saccharina latissima* across its latitudinal distribution gradient. *Front Mar Sci* submitted April 2021.

Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). *Mar Biol* 126:183–191.

Eckman JE, Duggins DO, Sewell AT (1989) Ecology of under story kelp environments. I. Effects of kelps on flow and particle transport near the bottom. *J Exp Mar Bio Ecol* 129:173–187.

Feehan C, Scheibling RE, Lauzon-Guay JS (2012) Aggregative feeding behavior in sea urchins leads to destructive grazing in a Nova Scotian kelp bed. *Mar Ecol Prog Ser* 444:69–83.

Fernandes F, Barbosa M, Oliveira AP, Azevedo IC, Sousa-pinto I, Valentão P, Andrade PB (2016) The pigments of kelps (Ochrophyta) as part of the flexible response to highly variable marine environments. *J Appl Phycol* 28:3689–3696.

Filbee-Dexter K, Wernberg T (2018) Rise of turfs: A new battlefront for globally declining kelp forests. *Bioscience* 68:64–76.

Filbee-Dexter K, Wernberg T, Grace SP, Thormar J, Fredriksen S, Narvaez CN, Feehan CJ, Norderhaug KM (2020) Marine heatwaves and the collapse of marginal North Atlantic kelp forests. *Sci Rep* 10:13388.

Fischer P, Brix H, Baschek B, Kraberg A, Brand M, Cisewski B, Riethmüller R, Breitbach G, Möller KO, Gattuso JP, Alliouane S, van de Poll WH, Witbaard R (2020) Operating cabled underwater observatories in rough shelf-sea environments: A technological challenge. *Front Mar Sci* 7:1–20.

Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgol Meeresunters* 34:15–29.

Foyer CH (2018) Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. *Environ Exp Bot* 154:134–142.

Franke K (2019) Performance of different life cycle stages of the Arctic kelps *Laminaria digitata* and *Saccharina nigripes* along temperature gradients. Master Thesis. University of Bremen.

Fredersdorf J, Müller R, Becker S, Wiencke C, Bischof K (2009) Interactive effects of radiation, temperature and salinity on different life history stages of the Arctic kelp *Alaria esculenta* (Phaeophyceae). *Oecologia* 160:483–492.

Hargrave MS, Foggo A, Pessarrodona A, Smale DA (2017) The effects of warming on the ecophysiology of two co-existing kelp species with contrasting distributions. *Oecologia* 183:531–543.

Hobday AJ, Alexander L V, Perkins SE, Smale DA, Straub SC, Oliver ECJ, Benthuysen JA, Burrows MT, Donat MG, Feng M, Holbrook NJ, Moore PJ, Scannell HA, Sen Gupta A, Wernberg T (2016) A hierarchical approach to defining marine heatwaves. *Prog Oceanogr* 141:227–238.

Hobday AJ, Oliver ECJ, Sen Gupta A, Benthuysen J., Burrows MT, Donat MG, Holbrook NJ, Moore PJ, Thomsen MS, Wernberg T, Smale DA (2018) Categorizing and naming marine heatwaves. *Oceanography* 31:162–173.

Van den Hoek C, Jahns HM, Mann DG (1993) Algen, Third Edi. Georg Thieme Verlag Stuttgart, New York.

Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed ecology and physiology, Second Edi. Cambridge University Press, Cambridge.

IPCC (2019) Summary for policymakers. In: *IPCC Special report on the ocean and cryosphere in a changing climate*. Pörtner H-O, Roberts DC, Masson-Delmotte V, Zhai P, Tignor M, Poloczanska E, Mintenbeck K, Alegría A, Nicolai M, Okem A, Petzold J, Rama B, Weyer NM (eds).

Kelly MW, Sanford E, Grosberg RK (2012) Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proc R Soc B Biol Sci* 279:349–356.

King NG, McKeown NJ, Smale DA, Moore PJ (2018) The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. *Ecography* 41:1469–1484.

Koch K, Thiel M, Tellier F, Hagen W, Graeve M, Tala F, Laeseke P, Bischof K (2015) Species separation within the *Lessonia nigrescens* complex (Phaeophyceae, Laminariales) is mirrored by ecophysiological traits. *Bot Mar* 58:81–92.

Leroi AM, Bennett AF, Lenski RE (1994) Temperature acclimation and competitive fitness: An experimental test of the beneficial acclimation assumption. *Proc Natl Acad Sci U S A* 91:1917–1921.

Li H, Monteiro C, Heinrich S, Bartsch I, Valentin K, Harms L, Glöckner G, Corre E, Bischof K (2020) Responses of the kelp *Saccharina latissima* (Phaeophyceae) to the warming Arctic: From physiology to transcriptomics. *Physiol Plant* 168:5–26.

Li H, Xu H, Zhang P, Gao M, Wang D, Zhao H (2017) High temperature effects on D1 protein turnover in three wheat varieties with different heat susceptibility. *Plant Growth Regul* 81:1–9.

Liesner D, Fouqueau L, Valero M, Roleda MY, Pearson GA, Bischof K, Valentin K, Bartsch I (2020a) Heat stress responses and population genetics of the kelp *Laminaria digitata* (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations. *Ecol Evol* 10:9144–9177.

Liesner D, Shama LNS, Diehl N, Valentin K, Bartsch I (2020b) Thermal plasticity of the kelp *Laminaria digitata* (Phaeophyceae) across life cycle stages reveals the importance of cold seasons for marine forests. *Front Mar Sci* 7:456.

Lüning K (1979) Growth strategies of three *Laminaria* species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). *Mar Ecol Prog Ser* 1:195–207.

Lüning K (1990) Seaweeds – Their environment, biogeography, and ecophysiology. Yarish C, Kirkman H (eds) Georg Thieme Verlag Stuttgart.

Lüning K (1984) Temperature tolerance and biogeography of seaweeds: The marine algal flora of Helgoland (North Sea) as an example. *Helgol Meeresunters* 28:305–317.

Martins N, Tanttu H, Pearson GA, Serrão EA, Bartsch I (2017) Interactions of daylength, temperature and nutrients affect thresholds for life stage transitions in the kelp *Laminaria digitata* (Phaeophyceae). *Bot Mar* 60:109–121.

Murchie EH, Lawson T (2013) Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. *J Exp Bot* 64:3983–3998.

Nepper-Davidsen J, Andersen DT, Pedersen MF (2019) Exposure to simulated heatwave scenarios causes long-term reductions in performance in *Saccharina latissima*. *Mar Ecol Prog Ser* 630:25–39.

Oliver ECJ, Donat MG, Burrows MT, Moore PJ, Smale DA, Alexander L V, Benthuysen JA, Feng M, Gupta AS, Hobday AJ, Holbrook NJ, Perkins-kirkpatrick SE, Scannell HA, Straub SC, Wernberg T (2018) Longer and more frequent marine heatwaves over the past century. *Nat Commun* 9:1324.

Pehlke C, Bartsch I (2008) Changes in depth distribution and biomass of sublittoral seaweeds at Helgoland (North Sea) between 1970 and 2005. *Clim Res* 37:135–147.

Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Ecology: Climate change and distribution shifts in marine fishes. *Science* 308:1912–1915.

Pörtner H., Lucassen M, Storch D (2005) Metabolic biochemistry: Its role in thermal tolerance and in the capacities of physiological and ecological function. *Fish Physiol* 22:79–154.

Pörtner HO, Farrell AP (2008) Physiology and climate change. *Science* 322:690–692.

Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315:95–97.

Provasoli L (1968) Media and prospects for the cultivation of marine algae. In: *Cultures and collections of algae*. *Proceedings of the Japanese Conference Hakone*. Japanese Society of Plant Physiology. Watanabe A, Hattori A (eds) Tokyo, p 63–75.

Reynolds RW, Smith TM, Liu C, Chelton DB, Casey KS, Schlax MG (2007) Daily high-resolution-blended analyses for sea surface temperature. *J Clim* 20:5473–5496.

Schlegel RW (2020) Marine heat wave tracker. http://www.marineheatwaves.org/tracker.

Schultze K, Janhke K, Krüß A, Weidemann W (1990) The macrofauna and macroflora associated with *Laminaria digitata* and *L. hyperborea* at the island of Helgoland (German Bight, North Sea). *Helgol Meeresunters* 44:39–51.

Simonson EJ, Scheibling RE, Metaxas A (2015) Kelp in hot water: I. Warming seawater temperature induces weakening and loss of kelp tissue. *Mar Ecol Prog Ser* 537:89–104.

Smale DA, Wernberg T (2013) Extreme climatic event drives range contraction of a habitat-forming species. *Proc R Soc B Biol Sci* 280:1–9.

Tatewaki M (1966) Formation of a crustaceous sporophyte with unilocular sproangia in *Scytosiphon lomentaria*. *Phycologia* 6:62–66.

Teagle H, Hawkins SJ, Moore PJ, Smale DA (2017) The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *J Exp Mar Bio Ecol* 492:81–98.

Terblanche JS, Deere JA, Clusella-trullas S, Janion C, Chown SL (2007) Critical thermal limits depend on methodological context. *Proc R Soc B Biol Sci* 274:2935–2942.

Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. *Curr Opin Plant Biol* 8:397–403.

Wernberg T, de Bettignies T, Joy BA, Finnegan PM (2016) Physiological responses of habitat-forming seaweeds to increasing temperatures. *Limnol Oceanogr* 61:2180–2190.

Wernberg T, Krumhansl K, Filbee-Dexter K, Pedersen MF (2018) Status and trends for the world's kelp forests, Second Edi. Elsevier Ltd.

Wiencke C, Bischoff B, Bartsch I, Peters AF, Breeman AM (1994) Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. *Bot Mar* 37:247–260.

Wiltshire KH, Kraberg A, Bartsch I, Boersma M, Franke H-D, Freund J, Gebühr C, Gerdts G, Stockmann K, Wichels A (2010) Helgoland Roads, North Sea: 45 Years of Change. *Estuaries and Coasts* 33:295–310.

Wright SW, Jeffrey SW, Mantoura RFC, Llewelly CA, Bjørnland T, Repeta D, Welschmeyer N (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar Ecol Prog Ser* 77:183–196.

Supplement

Statistics

Table S3.1: Results of the analysis of variance (ANOVA) to assess the effect of the fixed parameters temperature (T) and sampling time (ST) on the variability of survival, size and maximum quantum yield of photosystem II (F_v/F_m) of *Saccharina latissima* in the experiment.

			June 2018 : August 2018			August 2018 : August 2019		
	Parameter	numDF	denDF	*F* value	*p* value	denDF	*F* value	*p* value
survival	T	3	24	11.275	**< 0.001**	24	7.032	**< 0.001**
	ST	1	24	123.754	**< 0.001**	24	10.362	**< 0.001**
	T×ST	3	24	0.737	0.541	24	0.363	0.780
size	T	3	23	2.357	0.098	21	2.862	0.061
	ST	1	23	27.497	**< 0.001**	21	15.153	**< 0.001**
	T × ST	3	23	1.879	0.161	21	1.191	0.337
F_v/F_m	T	3	23	52.338	**< 0.001**	21	41.582	**< 0.001**
	ST	1	23	0.016	0.902	21	1.536	0.229
	T × ST	3	23	2.133	0.124	21	2.796	0.065

Note: Survival, size and F_v/F_m were tested against the interactive effects of temperature × sampling time, comparing June 2018 to August 2018 and August 2018 to August 2019. Tested values are the means of replicates (n = 3 – 4). numDF: numerator degrees of freedom; denDF: denominator degrees of freedom. Statistically significant values are marked in bold.

Table S3.2: Results of the analysis of variance (ANOVA) to assess the effect of the fixed parameters temperature (T) and sampling time (ST) on the variability of the chlorophyll *a* concentration (Chl *a*), accessory pigment concentration (Acc) and xanthophyll pigment concentration (VAZ) of *Saccharina latissima* in the experiment.

			June 2018 : August 2018			August 2018 : August 2019		
	Parameter	numDF	denDF	*F* value	*p* value	denDF	*F* value	*p* value
Chl *a*	T	4	27	2.058	0.114	28	5.488	**0.002**
	ST	1	27	0.971	0.333	28	3.942	0.057
	T × ST	3	27	4.276	**0.014**	28	1.983	0.125
Acc	T	4	27	1.239	0.318	28	4.999	**0.004**
	ST	1	27	5.111	**0.032**	28	2.817	0.057
	T × ST	3	27	5.850	**0.003**	28	1.446	0.245
VAZ	T	4	27	3.806	**0.014**	28	3.109	**0.031**
	ST	1	27	16.424	**< 0.001**	28	12.817	**0.001**
	T × ST	3	27	0.771	0.521	28	1.114	0.340

Note: Chl *a*, Acc and VAZ were tested against the interactive effects of temperature × sampling, comparing June 2018 to August 2018 and August 2018 to August 2019. Tested values are the means of replicates (n = 3 – 4). numDF: numerator degrees of freedom; denDF: denominator degrees of freedom. Statistically significant values are marked in bold.

Table S3.3: Results of the analysis of variance (ANOVA) to assess the effect of the fixed parameters temperature (T) and sampling time (ST) on the variability of the ratio of accessory pigments to the chlorophyll *a* concentration (Acc:Chl*a*), the ratio of the xanthophyll pool to the chlorophyll *a* concentration (VAZ:Chl*a*), the de-epoxidation state (DPS) of *Saccharina latissima* in the experiment.

			June 2018 : August 2018			**August 2018 : August 2019**		
	Parameter	numDF	denDF	*F* value	*p* value	denDF	*F* value	*p* value
Acc:Chl*a*	T	4	27	3.181	**0.029**	28	5.460	**0.002**
	ST	1	27	5.366	**0.028**	28	0.066	0.799
	T × ST	3	27	3.021	**0.047**	28	6.479	**< 0.001**
VAZ:Chl*a*	T	4	27	15.056	**< 0.001**	28	6.436	**< 0.001**
	ST	1	27	57.164	**< 0.001**	28	28.269	**< 0.001**
	T × ST	3	27	6.017	**0.003**	28	1.903	0.138
DPS	T	4	27	29.209	**< 0.001**	28	11.16	**< 0.001**
	ST	1	27	19.217	**< 0.001**	28	0.128	0.723
	T × ST	3	27	3.052	**0.046**	28	3.279	0.205

Note: Acc:Chl*a*, VAZ:Chl*a* and DPS were tested against the interactive effects of temperature × sampling, comparing June 2018 to August 2018 and August 2018 to August 2019. Tested values are the means of replicates (n = 3 – 4). numDF: numerator degrees of freedom; denDF: denominator degrees of freedom. Statistically significant values are marked in bold.

4. Publication III:

Coping with a changing Arctic: mechanisms of acclimation in the brown seaweed *Saccharina latissima* from Spitsbergen

Nora Diehl, Kai Bischof

published in
Marine Ecology Progress Series
January 2021

doi: 10.3354/meps13532

Title: Coping with a changing Arctic: Mechanisms of acclimation in the brown seaweed *Saccharina latissima* from Spitsbergen

Authors: Nora Diehl, Kai Bischof

Abstract

Polar regions are facing rapid temperature increase. In Arctic fjord systems, increased temperatures result in hyposalinity caused by the melting of sea ice and glaciers and freshwater run-off. Additionally, enhanced freshwater discharge and intrusion of nutrient-rich Atlantic water may result in nutrient input in summer. Combined, these factors might have a strong impact on primary producers, such as the abundant kelp species *Saccharina latissima*, an important foundation species in Arctic shallow-water coastal ecosystems. We ran two short-term two-factor experiments with field samples from Kongsfjorden (Svalbard) to evaluate the impact of temperature increase in summer combined with hyposalinity (temperature × salinity) or nutrient enrichment (temperature × nutrients) on the physiological and biochemical status of Arctic *S. latissima*. In the temperature × salinity experiment, growth and maximum photosynthetic quantum yield of photosystem II (F_v/F_m) were generally not affected. Temperature increase resulted in increased C:N ratios, based on decreasing nitrogen assimilation. Overall, hyposalinity had no severe effect but resulted in lower phlorotannin concentrations. Growth and F_v/F_m improved with increasing temperatures and nutrient enrichment. The de-epoxidation state of the xanthophyll cycle and mannitol declined at higher temperatures. Regarding other biochemical response variables, nutrients had no major impact (temperature × nutrients). In conclusion, in line with its broad latitudinal distribution range and adaptability, *S. latissima* proved to be highly resilient to changing abiotic drivers and will likely be promoted by warming in the future Arctic.

Introduction

Climate change is most pronounced in the polar regions. In the Arctic, temperature increase in the atmosphere is more than twice as fast as the global average (Meredith et al. 2019) and sea surface temperature (SST) is estimated to increase significantly towards the end of this century (Müller et al. 2009). Furthermore, marine heatwave events are proposed to increase in frequency, duration, intensity and spatial extent (Meredith et al. 2019). Kongsfjorden, on the west coast of Svalbard, is considered a 'hot spot' of climate change, since it is strongly impacted by warm off-shore Atlantic waters (Sundfjord et al. 2017). Hence, it is also regarded as a model system to study the impacts of change on high-Arctic fjord systems (Sundfjord et al. 2017, Bischof et al. 2019). Svendsen et al. (2002) still reported summer SSTs of about 4 °C. By 2012 the SST exceeded 6 °C (Dalpadado et al. 2016) and SST maxima of about 8 °C were measured in Kongsfjorden in the summer of 2019 (AWI 2019) and SST is expected to increase even further (Müller et al. 2009). Consequences of atmospheric temperature and SST increases include the loss of sea ice, glacier melting and enhanced freshwater run-off (e.g. Sundfjord et al. 2017). Between 1936 and 1999, the total freshwater inflow in the Arctic Ocean increased by 7 % (Bluhm & Gradinger 2008). In the Beaufort Gyre region freshwater content increased by 40 % between 2003 and 2017 (Meredith et al. 2019). In Kongsfjorden, absolute salinity (S_A) frequently decreases from 34 to 28 during the summer as a result of e.g. seasonal glacier and snow melt, river discharge or summer rainfall (Svendsen et al. 2002), and the temporary freshwater inflow can further intensify hyposaline conditions, resulting in an S_A of 23 and less down to 20 m water depth (Hanelt et al. 2001, Karsten et al. 2003, Karsten 2007).
In combination with rising temperatures and marine heatwave events in summer, salinity decrease or nutrient increase may have a strong interactive effect on marine organisms, mainly primary producers (e.g. Moy & Christie 2012, Diehl et al. 2020). Nutrient concentrations in Arctic fjords are relatively high in winter and early spring, inducing phytoplankton blooms as soon as sufficient light becomes available, which then lead to nutrient depletion from spring to fall (Aguilera et al. 2002, Bischof et al. 2019). N and P are essential macronutrients; N is available as nitrate, nitrite and ammonium and P as phosphate in marine environments. In Kongsfjorden, nitrate concentrations of 7 – 10 µM in winter and < 0.05 µM in summer are common, while phosphate concentration varies between 0.5 – 0.7 µM in winter and about 0.1 µM in summer (Rokkan Iversen & Seuthe 2011). N is considered the limiting factor for

macroalgal productivity, particularly in summer. Yet, enhanced freshwater discharge in summer and nutrient-rich Atlantic waters may increase nutrient supply in Arctic fjord systems (Gordillo et al. 2006, Zacher et al. 2009, Filbee-Dexter et al. 2019).

The rocky shores of Kongsfjorden are habitat for a rich seaweed community, dominated by kelp species, which are important foundation species providing food, shelter and nurseries for a multitude of associated organisms (e.g. Hop et al. 2016, Bischof et al. 2019). These canopy-forming brown macroalgae are exposed to a set of changing abiotic drivers, resulting in adjustments in the physiological and biochemical status, i.e. with respect to growth, photosynthesis, pigment content and C:N ratio (e.g. Conolly & Drew 1985, Davison et al. 1991, Gordillo et al. 2002, 2006, Fernandes et al. 2016, Nepper-Davidsen et al. 2019).

Kelps apply a range of protective mechanisms to acclimate to different environmental variations. Acclimation describes the physiological adjustment to abiotic conditions in the respective habitat, e.g. the improvement of photosynthetic efficiency to maximize performance (Hurd et al. 2014). Fernandes et al. (2016) described the general influence of environmental factors on pigment compositions as complex. Limited nitrate concentration, for instance, induced reduction in pigment content in kelps (Bartsch et al. 2008). The de-epoxidation state of the xanthophyll cycle (DPS) reduces intracellular stress triggered by reactive oxygen species e.g. at low temperatures (Wiencke & Bischof 2012) and thus, can be used as a general stress response. The nutrient supply in seaweeds can be surveyed with the C:N ratio. A low C:N ratio represents high N levels in the tissue and hence sufficient nutrient supply (Wiencke & Bischof 2012). The polyol mannitol acts as 'compatible solute' (Brown & Simpson 1972) and maintains intracellular functions under osmotic stress (Kirst 1989, Iwamoto & Shiraiwa 2005, Karsten 2012). It is the main photosynthetic product of Phaeophyceae (Iwamoto & Shiraiwa 2005) and is interconverted with the long-term C storage product laminarin (Yamaguchi et al. 1966, Kirst 1989, Karsten 2012, Graiff et al. 2016, Scheschonk et al. 2019). Phlorotannins (polyphenols) are multi-functional and play an ecological role in e.g. antifouling bioactivity and feeding deterrence, or act as antioxidants (Amsler 2008 and references therein). Temporal variations have also been found in response to changing abiotic factors, such as salinity and nutrient availability (Amsler 2008 and references therein).

To date, most studies have investigated the impact of environmental drivers on seaweeds in mono-factorial experiments (e.g. Davison & Davison 1987, Pfister & Van Alstyne 2003, Karsten 2007, Simonson et al. 2015, Nepper-Davidsen et al. 2019), while less is known about the combined interaction of several drivers (e.g. Henley & Dunton 1997, Pavia & Toth 2000,

Gordillo et al. 2002, Fredersdorf et al. 2009, Mandal et al. 2015). In recent studies on temperature-salinity interactions in kelps, Diehl et al. (2020) reported that the combination of abiotic drivers can have strong additive, but also antagonistic, effects on the photosynthetic and biochemical status of the endemic Arctic kelp *Laminaria solidungula* and Li et al. (2020) and Machado Monteiro et al. (2019) and Monteiro et al. (2020) investigated the effects of different temperatures and salinities on young *Saccharina latissima* sporophytes, reporting major changes on the transcriptomic level induced by both drivers. Growth, photosynthetic performance and pigment content are significantly reduced in Laminariales over summer, caused by seasonal nutrient depletion (Bartsch et al. 2008). Although the impact of nutrients on macroalgae in general is well investigated, only a few studies have focused on temperature-nutrient interactions (Mabin et al. 2013, Gao et al. 2016, Endo et al. 2017). Very little is known about the effects of nutrients in Arctic regions (Korb & Gerard 2000b, Gordillo et al. 2006, Filbee-Dexter et al. 2019), especially regarding interactions (Korb & Gerard 2000a). The individual effects of nutrients on macroalgae differ considerably among species, and no clear patterns in responses of macroalgae in general to variations in nutrient conditions can be determined (Gordillo et al. 2006). Filbee-Dexter et al. (2019) hypothesized that some Arctic kelp species can be physiologically limited by low nutrient levels and benefit from increasing nutrients.

Most Arctic kelp species have optimum growth between 10 and 15 °C and are expected to experience an increase in growth rates with increasing temperatures (Filbee-Dexter et al. 2019 and references therein). An increase in biomass and decrease in depth distribution of brown macroalgae have already been reported for Kongsfjorden (Müller et al. 2009, Bartsch et al. 2016). On the other hand, endemic species such as the kelp *L. solidungula* are reported to have limited growth and photosynthetic efficiency at temperatures above 10 °C (tom Dieck [Bartsch] 1992, Diehl et al. 2020).

One abundant kelp species in Kongsfjorden is *S. latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl et G.W. Saunders (Phaeophyceae, Laminariales), which grows between 1.5 and 16 m depth (Bischof et al. 2019). As a boreal-temperate species, *S. latissima* is found from polar (Svalbard) to warm-temperate (Portugal) regions (Araújo et al. 2016) and thrives at a temperature range between 0 and 23 °C (Müller et al. 2009 and references therein). It tolerates oligotrophic conditions and can survive hyposaline conditions down to S_A 8 (Bartsch et al. 2008). *Saccharina latissima* is proposed to have temperature ecotypes, which are adapted to Arctic conditions (Müller et al. 2009). With increasing temperatures, a global northward shift

of *S. latissima* is expected, assuming that it will be able to settle at even higher latitudes, provided that rocky substrates are available (Müller et al. 2009). Between 1996/1998 and 2012/2013, *S. latissima* in Kongsfjorden already experienced a slight increase in biomass and a slight reduction in depth extension (Bartsch et al. 2016).

A continuous change in Arctic shallow-water benthic community structure is expected with progressing temperature increase (Krause-Jensen & Duarte 2014). Müller et al. (2009) predicted major ecological consequences on local phytobenthic communities in polar environments due to climate change and stressed the importance of further investigations on the complexity of temperature increase and multiple interacting factors. *Saccharina latissima* has a broad distribution range and is suggested to employ opportunistic growth strategies (Bartsch et al. 2008). Several studies have reported species with narrow geographical distributions being less tolerant to environmental changes than species with wide distributions (Kelly et al. 2012 and references therein, Sunday et al. 2015). Reich et al. (2015) proposed that boreal-temperate species, such as *S. latissima*, most probably will have neutral or positive responses to future warming. However, local adaptation such as the evolution of ecotypes may play important roles in determining environmental tolerances of wide-ranging species, especially at the edge of the species range (Kelly et al. 2012, Reich et al. 2015).

In order to investigate the sensitivity of Arctic populations, we conducted two short-term two-factor experiments (temperature × salinity, temperature × nutrients) with *S. latissima* collected from Kongsfjorden, Svalbard. While most studies on environmental drivers apply rather extreme conditions of the abiotic factors, we wanted to shed light on a more realistic scenario. For that purpose, the set-up was based on possible future climate change and marine heatwave scenarios in the Arctic (Hobday et al. 2016, Meredith et al. 2019). The temperatures (4, 6, 8, 10 °C) were applied according to reported Arctic summer temperatures (Svendsen et al. 2002, Wiencke et al. 2007, Dalpadado et al. 2016, AWI 2019). Hyposalinity was based on minimum S_A 23 – 28 in Kongsfjorden over summer (Hanelt et al. 2001, Svendsen et al. 2002) and nutrient treatments were based on the highest possible concentrations over the year (winter) (Rokkan Iversen & Seuthe 2011).

In line with its wide acclimation capabilities to different environmental variations and the supportive effects of enhanced nutrient concentrations on seaweeds (e.g. Gerard 1997, Bischof et al. 1998, Karsten 2007, Bartsch et al. 2008, Hurd et al. 2014), we expected *S. latissima* to benefit from short-term temperature increase and nutrient enrichment, rather than being harmed by hypoosmotic conditions.

Material and methods

Sampling and preparation

SCUBA divers collected *Saccharina latissima* (Linnaeus) (Lane et al. 2006) from Kongsfjorden, Svalbard in late June and early July 2019. Samples were taken from ~ 12 m water depth from the same location ('Old Pier,' Ny-Ålesund, 78° 55.4956' N, 11° 55.1088' E). All sporophyte blades had a length of 1 – 1.80 m. For both experiments, we cut discs (2.3 – 2.4 cm diameter) from the meristem and equally distributed them to the different treatments and replicates – 10 discs of five individuals were grouped in each of 4 replicates. For wound healing, we maintained the samples in aerated 2-L clear plastic bottles at 4 °C for two days with ½ Provasoli enriched seawater (½ PES; Provasoli 1968; modifications: HEPES-buffer instead of TRIS, double concentration of Na_2glycerophosphate, iodine enrichment after Tatewaki 1966) and a 24 h artificial photon fluence rate of 30 µmol m^{-2} s^{-1} (Econlux, SolarStringer LED SunStrip 'daylight'). This irradiance was reported to be the average daily irradiance at depths of 1 – 5 m in summer (Bischof et al. 2002). After wound healing, we shock-froze discs in liquid N_2 for the initial sampling and stored them at −80 °C (t_0, where t = days). After wound healing, we acclimated the initial samples (t_0) to four different temperatures (4, 6, 8, 10 °C) with an increase of 2 °C every second day.

Experimental set-up

Temperature × salinity

The salinity treatments started after temperature acclimation (t_6), using untreated filtered fjord seawater (S_A 32 – 34) as the control treatment. For the hyposaline treatment (low: S_A 25), we diluted filtered fjord seawater with tap water. Tap water in Ny-Ålesund is taken from a nutrient-poor meltwater lake. We kept the samples in aerated 2-L clear plastic bottles at 30 µmol m^{-2} s^{-1} constant light during the entire period of the experiment and in ½ PES, to ensure sufficient nutrient supply in both treatments. We exchanged water twice a week. After eight days of exposure to the two-factor treatment (t_{14}), we shock-froze all samples in liquid N_2, stored them at −80 °C and freeze-dried them before biochemical analyses. At 8 and 10 °C a diatom contamination became visible after one week.

Temperature × nutrients

During the temperature acclimation phase, we maintained the samples in ½ PES (S_A 32 – 34) to ensure sufficient nutrient supply, even though a ½ PES treatment is much higher than what *S. latissima* experiences in nature. The nutrient treatments started after temperature acclimation (t_6). For the control seawater (SW) treatment, we used filtered seawater from the fjord which has ~ 0.05 µM nitrate (NO_3^-) and 0.1 µM phosphate (PO_4^{3-}) in summer (Bischof et al. 2019). We provided the nutrient treatment by adding 10 µM NO_3^- and 1 µM PO_4^{3-} to filtered seawater (SW+N+P), based on reported winter nutrient concentrations (Bischof et al. 2019). Even though we used SW+N+P as the nutrient-enriched treatment, the nutrient concentration is far lower than in PES treatments. We changed water every day and kept the samples in aerated 2-L clear plastic bottles at 30 µmol m^{-2} s^{-1} constant light and at S_A 32 – 34 during the entire period of the experiment. After eight days of two-factor treatment (t_{14}), we shock-froze all samples in liquid N_2, stored them at −80 °C, and freeze-dried them before biochemical analyses.

Response variables

Physiological parameters

To monitor the physiological response variables, we measured the size and the maximum photosynthetic quantum yield of the discs every second day (t_8 – t_{14}), as well as before and after the temperature acclimation (t_0, t_6).

We photographed the discs using a 3 × 3 cm square as a size reference and analyzed the pictures with ImageJ (Version 1.52a, Java 1.8.0_112). To compare algal growth in the different treatments, we adjusted the initial size to 100 % and calculated size of each sample as % of initial.

The maximum quantum yield of photosystem II (F_v/F_m) is a proxy for photosynthetic efficiency and gives an indication of the physiological status of the alga (Hurd et al. 2014). Therefore, we determined the *in vivo* chlorophyll *a* fluorescence of each sample after 10 min of dark acclimation using an Imaging-PAM chlorophyll fluorometer (Walz Mess- und Regeltechnik).

For a better comparison of the treatments, we additionally adjusted each initial F_v/F_m to 100 % reference and calculated F_v/F_m of the treatments as % of initial, after *arcsin* transformation.

Pigments

We analyzed photosynthetic and accessory pigments following Koch et al. (2015). In short, we extracted 50 – 150 mg of freeze-dried samples in 1 mL 90 % aqueous acetone (volume percent; v/v) in darkness at 4 °C for 24 h. We filtered the supernatant and analyzed it via HPLC (LaChromElite® system, L-2200 autosampler [chilled], DA-detector L-2450; VWR-Hitachi Internationa), applying a gradient according to Wright et al. (1991), and separating by a Spherisorb® ODS-2 column (250 × 4.6 mm, 5 µm; Waters). We used standards of chlorophyll *a* and *c*2, fucoxanthin, violaxanthin, antheraxanthin, and zeaxanthin (DHI Lab Products) to identify and quantify the peaks. Chlorophyll *a* (Chl *a*), the accessory pigment pool (Acc) and the pool size of the xanthophyll cycle (VAZ = violaxanthin [V], antheraxanthin [A], zeaxanthin [Z]) are expressed in µg g^{-1} dry weight (DW). Variations in pigment content or composition an be indicative of adjustments to environmental conditions (Bartsch et al. 2008). Therefore, we calculated the Acc:Chl*a* ratio. The xanthophyll cycle is a mechanism to reduce the generation of reactive oxygen species, which are mainly induced by high light stress but also promoted at low temperatures (Dring 2005). DPS describes the process in which violaxanthin is converted to zeaxanthin, reducing intracellular stress (Wiencke & Bischof 2012). Thus, DPS can be used as a general stress response variable for macroalgae. We calculated the Acc:Chl*a* ratio and calculated the DPS after Colombo-Pallotta et al. (2006):

$$DPS = \frac{(Z+0.5A)}{V+A+Z} \quad \textbf{(1)}$$

C:N ratio, carbon and nitrogen

The C:N ratio gives an overview on the nutrient supply in seaweed. We analyzed the C:N ratios, total C and total N content following the protocol of Graiff et al. (2015). In short, we weighed 2 mg of ground samples into tin cartridges. The samples were combusted at 950 °C and the content of total C and N was analyzed in an elemental analyzer (Vario EL III, Elementar). We used acetanilide (C_8H_9NO) as a standard (Verardo et al. 1990) and expressed the total C and total N content in mg g^{-1} DW.

Mannitol

Mannitol is not only the main photosynthetic product in brown algae, it also compensates for osmotic stress by adjusting to external salinity (Kirst 1989, Iwamoto & Shiraiwa 2005, Karsten 2012). We determined the mannitol concentration after Diehl et al. (2020), using the method described by Karsten et al. (1991). In short, we extracted 10 – 15 mg of freeze-dried

sample in 1 mL aqueous ethanol (70 %, v/v) and incubated it in a water bath at 70 °C for 3 – 4 h. The supernatant was portioned into aliquots of 800 µL, evaporated to dryness and re-dissolved in 800 µL 100 % dH_2O. We analyzed the samples in an HPLC system (Agilent Technologies system 1200 Series) with an Aminex Fast Carbohydrate Analysis Column HPAP (100 × 7.8 mm, 9 µm, BioRad), protected by a guard cartridge (Phenomenex, Carbo-Pb-2+ 4 × 3.00 mm i.d.) and 100 % dH_2O as mobile phase. We calculated mannitol contents in mg g^{-1} DW, using purified D(-)-mannitol standards ($C_6H_{14}O_6$, Roth) for calibration (1, 6 and 10 mM).

Phlorotannins

Phlorotannins are multi-functional and are an important protective mechanism against a variety of abiotic factors (Amsler 2008 and references therein). Following Springer et al. (2017), we determined the total phlorotannin content using the Folin-Ciocalteu method described by Cruces et al. (2012). In short, we extracted 12 – 15 mg of freeze-dried samples in 1 mL of 70 % acetone (v/v) for 24 h at 4 °C in darkness with constant shaking. To each extract, we added 250 µL dH_2O, 200 µL 20 % sodium carbonate (Na_2CO_3) and 100 µL 2N Folin-Ciocalteu reagent (Sigma-Aldrich). After 45 min of incubation, we read the absorbance at λ 730 nm in a microplate reader (FLUOstar OPTIMA; BMG Labtech). We calculated the total soluble phlorotannin concentrations in mg g^{-1} DW by using purified phloroglucinol ($C_6H_6O_3$; Sigma-Aldrich) (0, 25, 50, 75, 100, 125, 150, 200, 250, 300 µg mL^{-1}) for calibration.

Statistical analyses

We excluded outliers (R-Student Bonferroni, $p < 0.05$) from further analyses. For each data-set, we executed a Shapiro-Wilk test (normal distribution; $p > 0.05$) and Levene's test (homogeneity of variance; $p > 0.05$) and transformed the data if required.

We used repeated measures two-way ANOVA to analyze growth, as increase in size (% of initial), and F_v/F_m (% of initial), followed by a post hoc Tukey's test to reveal significant differences. We ran generalized linear models (GLMs) with a Gaussian distribution on the biochemical response variables for within-subjects effects (in treatments). For between-subjects effects (between two treatments and initial), we tested the data-sets using one-way ANOVA, with a post hoc Tukey's test. In the case that pre-requirements were not fulfilled, we applied Kruskal-Wallis tests, followed by the Dunn-Bonferroni's post hoc test. The level of significance was set to $\alpha = 0.05$. We used RStudio (Version 1.2.1335, 2019) for all statistical analyses and

box plots. Results from statistical analyses are presented in **Tables S4.1 – S4.12** in **Supplement 1**.

Results

Temperature × salinity

Size increased significantly over time ($F_4 = 97.45$, $p < 0.001$) (**Fig. 4.1a**). Temperature ($F_3 = 5.35$, $p < 0.01$), salinity ($F_1 = 5.59$, $p < 0.05$) and the temperature × salinity interaction ($F_3 = 15.28$, $p < 0.001$) all revealed mathematically significant impacts. However, comparing the individual treatments with each other, detected differences could not be assigned to any of the stress treatments, i.e. neither low salinity at 4 °C (low/4 °C) and control salinity at 10 °C (control/10 °C), nor control/4 °C and low/10 °C differed from each other.

Salinity and temperature both did not affect F_v/F_m (**Fig. 4.2a**) throughout the experiment and no interactive effect was found. Nevertheless, almost all treatments resulted in higher F_v/F_m values than at the beginning of the experiment ($F_4 = 5.14$, $p < 0.001$). Absolute values of F_v/F_m are summarized in the electronic supplements in **Table S4.13a** in **Supplement 2**.

Temperature or salinity changes overall did not affect the Chl *a*, Acc or VAZ, the Acc:Chl*a* ratio or DPS (**Table 4.1a**). However, the control/10 °C sample revealed significantly lower Chl *a* content than the initial sample (GLM: $\chi^2_3 = 8.87$, $p < 0.05$).

Different salinities had no impact on the C:N ratio (**Fig. 4.3a**), although the C:N ratio of control and low-salinity treatments were lower than the initial ratio ($F_{(Temp)4} = 13.00$, $p_{(Temp)} < 0.001$; $F_{(Sal)2} = 13.88$, $p_{(Sal)} < 0.001$). A trend to increasing C:N ratios with increasing temperatures was observable and more distinct in the control treatment. These results were not based on differences in total C content (**Fig. S4.1a** in **Supplement 2**) but entirely based on changes in total N content (**Fig. 4.3b**). The total N content decreased at both salinities between 4 and 10 °C ($F_4 = 6.33$, $p < 0.01$). Although the initial C:N ratio was significantly different from almost all treatments, the initial N content did not differ from low salinity at 6, 8 or 10 °C or control salinity at 10 °C.

No differences in mannitol concentration (**Fig. 4.4a**) were detected within the temperature and the salinity treatments at the end of the experiment. However, the initial value was higher than the end values (Kruskal-Wallis: $\chi^2_{4(Temp)} = 24.50$, $p_{(Temp)} < 0.001$; $\chi^2_{2(Sal)} = 21.29$, $p_{(Sal)} < 0.001$). Since the data-set was non-parametric, it could not be tested for interactions. Overall mannitol concentrations varied between (mean ± SD) 109 ± 3 and 388 ± 27 mg g^{-1} DW.

Phlorotannin content (**Fig. 4.5a**) was affected by the two different salinities, eventually resulting in lower concentrations in the low salinity treatment (F_2 = 37.03, p < 0.001). However, the concentrations at the end of the experiment (low and control salinity) were higher than the initial values (control salinity; GLM: χ^2_1 = 30.29, p < 0.001). Overall, temperature had no significant impact on the phlorotannin concentration, but the initial sample also differed from the temperature treatments, except low/4 °C and low/6 °C (F_4 = 9.46, p < 0.001). An interaction of salinity and temperature was not detected.

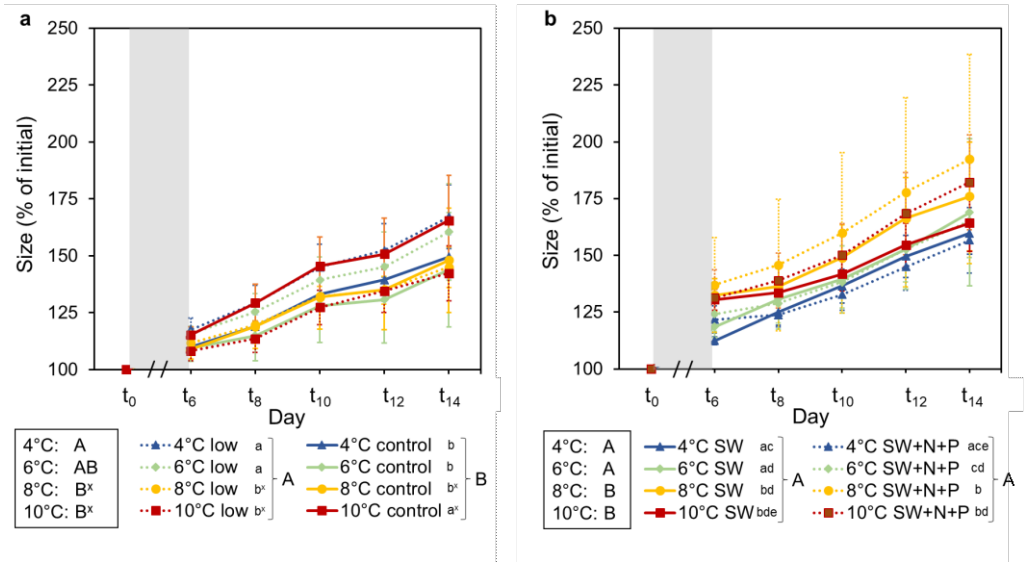


Fig. 4.1: Size of *Saccharina latissima* during the two-factor exposure at four temperatures (4, 6, 8, 10 °C) and **a:** salinity treatments (low, control) or **b:** nutrient treatments (SW+N+P: seawater + nitrate + phosphate). Gray shading indicates the temperature acclimation phase (t_0 – t_6). Values are means ± SD (n = 4). ˣ marks diatom contamination. Significances are marked by different letters; lowercase letters mark within-subjects effects and uppercase letters between-subjects effects (repeated two-way ANOVA with post hoc Tukey's test; p < 0.05).

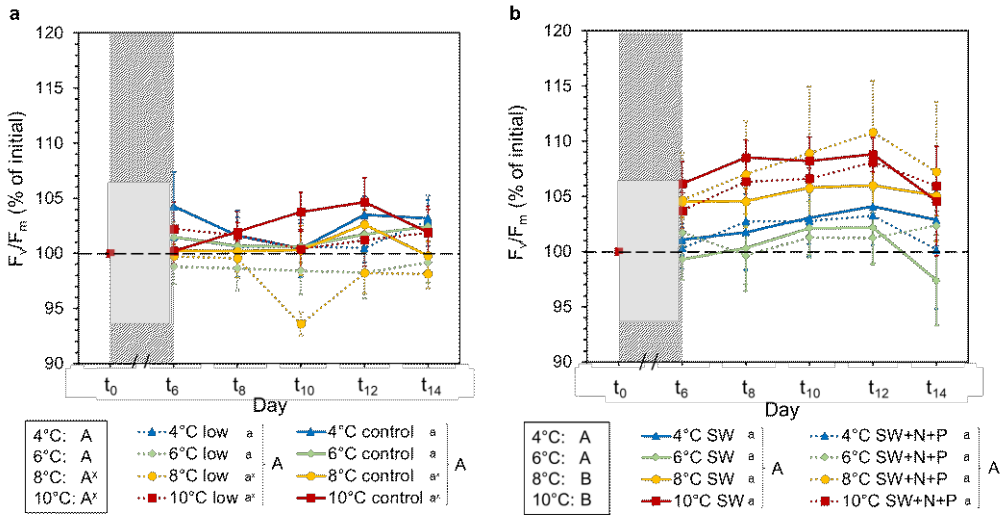


Fig. 4.2: Maximum quantum yield of photosystem II (F_v/F_m) of *Saccharina latissima* during the two-factor treatment at four temperatures (4, 6, 8, 10 °C) and **a:** salinity treatments (low, control) or **b:** nutrient treatments (SW+N+P: seawater + nitrate + phosphate). The dashed horizontal line represents the initial values of 100 %. Other details as in **Fig. 4.1**.

Temperature × nutrients

The samples grew significantly (F_4 = 61.68, p < 0.001) throughout the experiment (**Fig. 4.1b**). Overall, the different nutrient treatments did not affect the size of the samples, but temperature had an impact (F_3 = 17.34, p < 0.001), resulting in larger discs at higher temperatures, especially in the SW+N+P treatments, even though significant interactions could not be detected.

The results of F_v/F_m were comparable to results from the growth analysis (**Fig. 4.2b**). While the nutrient treatments had no general effect, an effect of temperature was detected (F_3 = 43.49, p < 0.001). At 8 and 10 °C, samples had higher F_v/F_m values than at 4 and 6 °C. The combination of additional nutrient concentrations and higher temperatures (8 and 10 °C) apparently led to stronger F_v/F_m increases than temperature increase alone (F_3 = 3.00, p < 0.05). Almost all treatments resulted in higher F_v/F_m values than at the beginning of the experiment (F_4 = 5.46, p < 0.001). Absolute values of F_v/F_m are summarized in **Table S4.13b**.

The pigment analyses did not show any trends or significant differences in temperature or nutrient treatments in the absolute pigment concentration and Acc:Chl*a* ratio (**Table 4.1b**). However, with respect to temperature, DPS significantly decreased between 4 and 6 to 10 °C (GLM: χ^2_3 = 24.50, p < 0.01).

In contrast to the temperature × salinity experiment, neither the C:N ratio (**Fig. 4.3c**), nor total N content (**Fig. 4.3d**) or total C content (**Fig. S4.1b**) overall showed significant effects from the temperature or nutrients treatment.

Nutrient treatments had no impact on mannitol content (**Fig. 4.4b**), while temperature did (Kruskal-Wallis: χ^2_4 = 30.56, p < 0.001). In the SW+N+P treatment, the 10 °C samples contained significantly less mannitol than at 4, 6 and 8 °C (F_4 = 7.73, p < 0.001), but did not significantly differ from SW/10 °C. Since the data-set was non-parametric, it could not be tested for interactions. Nonetheless, 10 °C, especially paired with high nutrients, may have had an impact on the mannitol concentration.

Overall, temperature had no effect on phlorotannin concentration (**Fig. 4.5b**). Even though no significance between the two nutrient treatments was determined, a trend to higher phlorotannin concentration at lower nutrient concentrations was evident, which is intensified at higher temperatures (GLM: χ^2_4 = 10.21, p < 0.05). The initial samples (½ PES) contained significantly less phlorotannins, than both experimental treatments (Kruskal-Wallis: $\chi^2_{4(Temp)}$ = 19.53, p < 0.001; $\chi^2_{2(Nut)}$ = 15.25, p < 0.001).

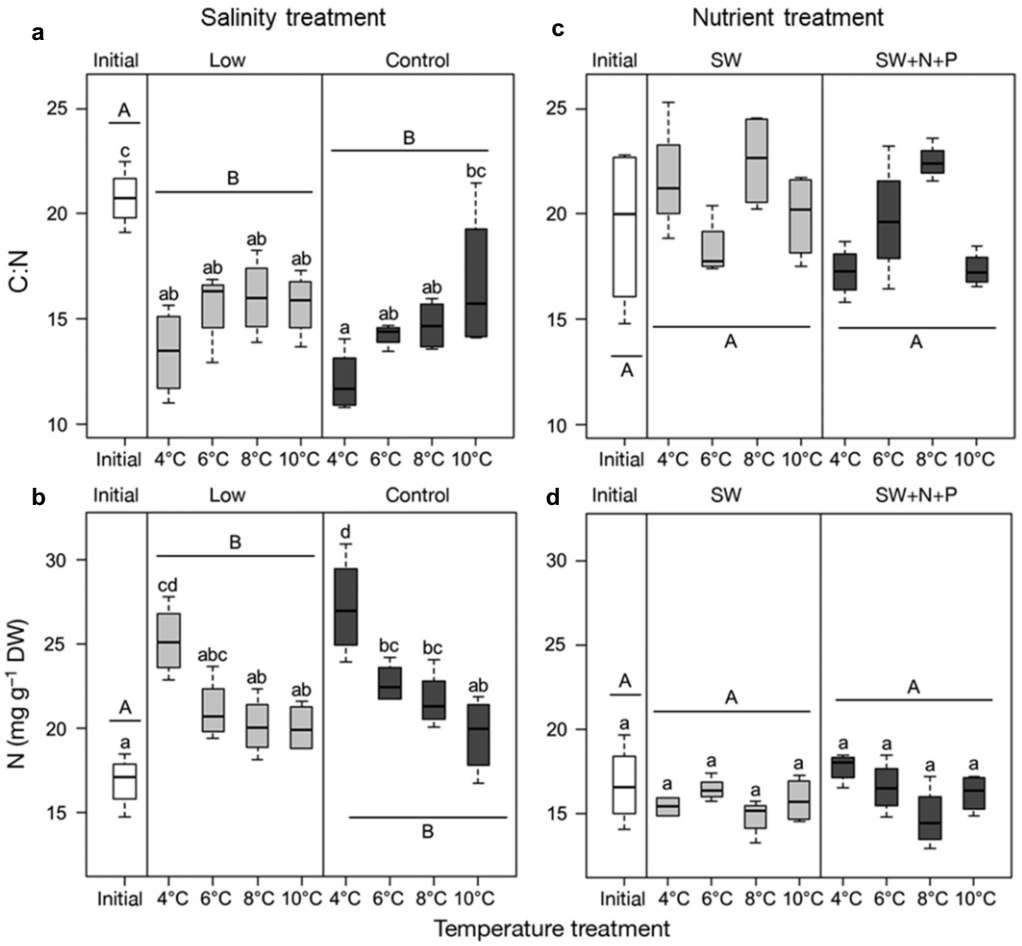


Fig. 4.3 a & c: C:N ratio and **b & d:** total N content in *Saccharina latissima* after one week of two-factor treatment at four temperatures (4, 6, 8, 10 °C) and salinity treatments (low, control) (**a & b**) or nutrient treatments (SW+N+P: seawater + nitrate + phosphate) (**c & d**). The boxplots represent the median (50th percentile), the interquartile range (25th to the 75th percentile) and the minimum and maximum values of the data-sets. Significant differences are marked with different letters; lowercase letters mark within-subjects effects and uppercase letters between-subjects effects (n = 4; a, b, d: two-way ANOVA with post hoc Tukey's test; c: Kruskal-Wallis test with post hoc Dunn-Bonferroni's test; $p < 0.05$).

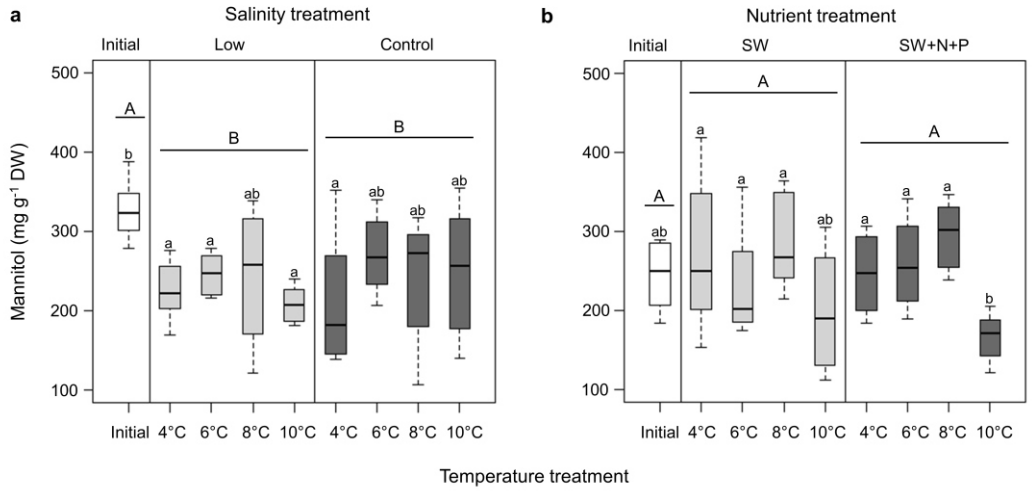


Fig. 4.4: Mannitol concentration in *Saccharina latissima* after one week of two-factor treatment with four temperatures (4, 6, 8, 10 °C) and **a:** salinity treatments (low, control) or **b:** nutrient treatments (SW+N+P: seawater + nitrate + phosphate). The boxplots represent the median (50th percentile), the interquartile range (25th to the 75th percentile) and the minimum and maximum values of the data-sets. Significant differences are marked with different letters; lowercase letters mark within-subjects effects and uppercase letters between-subjects effects (n = 4; two-way ANOVA with post hoc Tukey's test; $p < 0.05$).

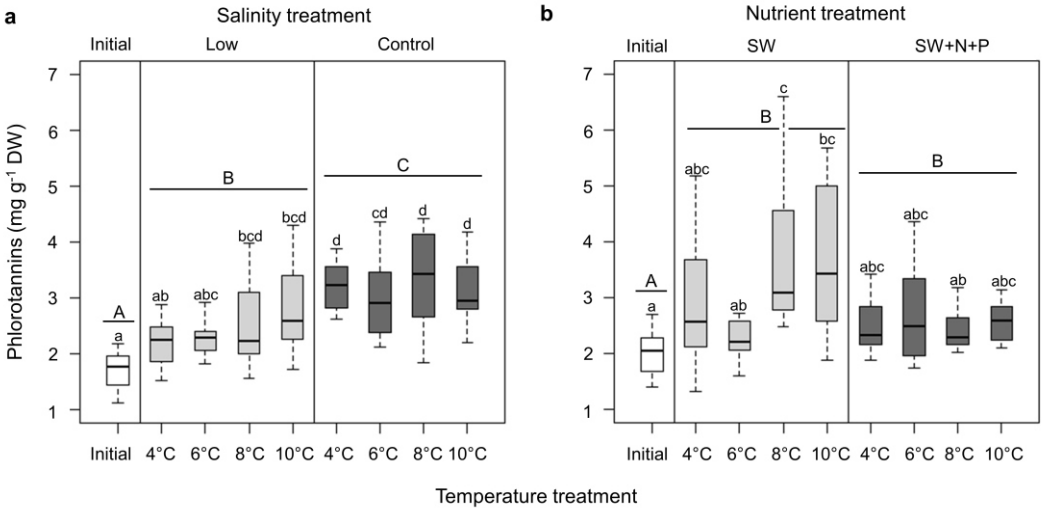


Fig. 4.5: As in **Fig. 4.4**, but for phlorotannin concentration.

Table 4.1: Absolute pigment concentrations for Chl *a*, accessory pigments (Acc) and the pool of xanthophyll cycle pigments (VAZ: violaxanthin, antheraxanthin, zeaxanthin); Acc:Chl*a* ratio; and de-epoxidation state of the xanthophyll cycle (DPS) in *Saccharina latissima* after one week of two-factor treatment at four temperatures (4, 6, 8, 10 °C) and **a:** salinity treatments (low, control) or **b:** nutrient treatments (SW+N+P: seawater + nitrate + phosphate). Values are means ± SD (n = 4). [x] marks diatom contamination. Significant differences are marked with different letters (generalized linear model; $p < 0.05$). DW: dry weight.

	Treatments		Chl *a* (µg g^{-1} DW)		Acc (µg g^{-1} DW)		VAZ (µg g^{-1} DW)		Acc:Chl*a*		DPS	
	Initial		1041.5 (± 207.9)	a	818.9 (± 136.5)	a	72.6 (± 14.4)	a	0.72 (± 0.09)	a	0.038 (± 0.003)	a
(a) Temperature × Salinity	4 °C	Low	922.1 (± 84.4)	ab	755.1 (± 72.0)	a	82.1 (± 12.1)	a	0.82 (± 0.02)	a	0.023 (± 0.004)	a
		Control	866.1 (± 324.8)	ab	729.3 (± 198.4)	a	86.5 (± 24.2)	a	0.83 (± 0.05)	a	0.027 (± 0.006)	a
	6 °C	Low	822.8 (± 234.8)	ab	640.6 (± 158.0)	a	86.8 (± 20.2)	a	0.80 (± 0.02)	a	0.022 (± 0.005)	a
		Control	592.6 (± 197.3)	ab	490.3 (± 145.1)	a	51.9 (± 21.6)	a	0.82 (± 0.03)	a	0.032 (± 0.013)	a
	8 °C	Low[x]	591.5 (± 199.7)	ab	488.1 (± 145.0)	a	65.0 (± 20.1)	a	0.85 (± 0.03)	a	0.033 (± 0.007)	a
		Control[x]	630.1 (± 176.1)	ab	566.2 (± 139.0)	a	78.4 (± 21.1)	a	0.88 (± 0.02)	a	0.023 (± 0.013)	a
	10 °C	Low[x]	657.6 (± 316.3)	ab	606.9 (± 210.4)	a	79.7 (± 29.4)	a	0.89 (± 0.05)	a	0.021 (± 0.004)	a
		Control[x]	447.9 (± 147.8)	b	387.0 (± 78.1)	a	46.9 (± 4.5)	a	0.86 (± 0.11)	a	0.025 (± 0.006)	a
	Initial		577.0 (± 151.1)	a	373.4 (± 83.3)	a	39.3 (± 7.3)	a	0.64 (± 0.02)	a	0.149 (± 0.073)	a
(b) Temperature × Nutrients	4 °C	SW	514.3 (± 64.1)	a	490.34 (± 38.8)	ab	59.1 (± 8.6)	a	0.95 (± 0.02)	b	0.019 (± 0.020)	ab
		SW+N+P	772.4 (± 322.4)	a	721.8 (± 224.1)	b	84.7 (± 32.0)	a	0.94 (± 0.05)	b	0.026 (± 0.021)	ab
	6 °C	SW	703.0 (± 185.7)	a	631.2 (± 132.0)	ab	80.3 (± 19.9)	a	0.91 (± 0.06)	b	0.037 (± 0.012)	ab
		SW+N+P	636.6 (± 216.4)	a	613.5 (± 160.4)	ab	70.3 (± 11.7)	a	0.99 (± 0.05)	b	0.022 (± 0.007)	ab
	8 °C	SW	710.0 (± 32.6)	a	685.6 (± 32.2)	ab	89.1 (± 10.7)	a	0.92 (± 0.03)	b	0.012 (± 0.004)	b
		SW+N+P	609.7 (± 227.8)	a	611.6 (± 191.5)	ab	77.0 (± 29.0)	a	1.01 (± 0.04)	b	0.016 (± 0.003)	ab
	10 °C	SW	643.0 (± 228.9)	a	627.3 (± 143.5)	ab	82.5 (± 17.7)	a	0.97 (± 0.06)	b	0.020 (± 0.006)	ab
		SW+N+P	661.4 (± 290.8)	a	626.9 (± 181.6)	ab	71.0 (± 9.7)	a	0.95 (± 0.06)	b	0.012 (± 0.007)	b

Discussion

Temperature increase and marine heatwave events are severe in the Arctic (Meredith et al. 2019) and likely impact the marine environment (Smale et al. 2019). While moderate nutrient enrichment usually implies positive and beneficial impacts on primary producers (e.g. Gerard 1997, Henley & Dunton 1997, Figueroa et al. 2009, Gao et al. 2016), salinity variations often result in physiological stress in Arctic seaweeds (Karsten 2007, Fredersdorf et al. 2009, Diehl et al. 2020). By regulating their physiological and biochemical status, seaweeds can acclimate to environmental variations and thereby reduce stress or maximize their performance. In our study, field sporophytes of Arctic *Saccharina latissima* revealed only minor effects of the interaction of temperature × salinity or temperature × nutrients. Overall, temperature increase caused physiological and biochemical changes, while the hyposalinity and nutrient enhancement had no major impact (**Table 4.2**).

As reported before, by approaching the optimum temperature range of polar specimens, such as *S. latissima* in our study, higher temperature had a beneficial impact on the physiological performance and may have even compensated for the incurred biochemical stress (Rautenberger & Bischof 2006). In our study, hyposalinity did not impair *S. latissima*, neither on a physiological nor on a biochemical level. Nutrient depletion and enrichment are known to affect macroalgal production (Hurd et al. 2014); however, we could not detect major improvements of nutrient addition on in the physiological and biochemical status after addition of nutrients.

Table 4.2: Summary of the abiotic factors, their respective impact on physiological and biochemical response variables and resulting effects on overall performance of Arctic *Saccharina latissima*: **a:** temperature × salinity experiment, **b:** temperature × nutrients experiment. ↑ = response variable increased, ↓ = response variable decreased, ↔ = no change. Resulting effects on algal performance: / = neutral, + = beneficial, − = adverse. [x] = diatom contamination in 8 and 10 °C treatments. ½ PES: ½ Provasoli-enriched seawater; S_A: absolute salinity; other abbreviations as in **Table 4.1**.

	(a) Temp × Sal [x]			**(b) Temp × Nut**		
	Set-up: ½ PES 30 µmol m^{-2} s^{-1} Treatments: 4, 6, 8, 10 °C low: S_A 25 control: S_A 32 – 34			Set-up: S_A 32 – 34 30 µmol m^{-2} s^{-1} Treatments: 4, 6, 8, 10 °C SW: seawater SW+N+P: seawater + 10 µM NO_3^- + 1 µM PO_4^{3-}		
Response variable	**Increasing temp**	**Hyposalinity**	**Temp × Sal**	**Increasing temp**	**Enhanced Nut**	**Temp × Nut**
growth	↔ /	↔ /	↔ /	↑ +	↔ /	↔, ↑ trend /
F_v/F_m	↔ /	↔ +	↔ /	↑ +	↔ /	↑ +
pigments (Chl *a*, Acc, VAZ, Acc:Chl*a*)	↔ /	↔ +	↔ /	↔ /	↔ /	↔ /
DPS	↔ /	↔ +	↔ /	↓ +	↔ /	↔ /
C:N	↔, ↑ trend /	↔ +	↔, ↑ trend /	↔ /	↔ /	↔ /
total C	↔ /	↔ +	↔ /	↔ /	↔ /	↔ /
total N	↓ +	↔ +	↔ /	↔ /	↔ /	↔ /
mannitol	↔ /	↔ +	not tested	↓ +	↔ /	not tested
phlorotannins	↔ /	↓ +	↔ /	↔ /	↔, ↑ trend /	not tested, ↑ trend

Mechanisms of acclimation responses

Saccharina latissima, as well as other kelp species are physiologically affected by variations in temperature, salinity and nutrients and therefore apply a variety of mechanisms for protection against negative effects caused by these variations. As reported in previous studies, within the respective tolerance range, enhanced temperatures had a beneficial impact on growth and F_v/F_m of Arctic *S. latissima* (e.g. Iñiguez et al. 2016, Li et al. 2020). Approaching 10 °C, which is within the optimum temperature range of 10 – 15 °C (Müller et al. 2009), growth and F_v/F_m increased, while at lower temperatures both response variables declined significantly (Fortes & Lüning 1980, Davison et al. 1991, Müller et al. 2009). Bartsch et al. (2008) summarized the genus *Laminaria sensu lato* as rather stenohaline, with respect to growth and photosynthesis. *Saccharina latissima* exhibits optimum growth between S_A 23 and 31, while F_v/F_m tolerates even broader ranges of S_A 25 – 55 (Gerard et al. 1987, Karsten 2007). In contrast to Li et al. (2020), we could not detect any impact of salinity on growth of Arctic *S. latissima*. In accordance to earlier studies, we also detected no impact of hyposalinity in F_v/F_m and all samples could be considered as healthy (Dring et al. 1996, Karsten 2007, Li et al. 2020). The increase of F_v/F_m over time, can be explained by the optimal cultivation conditions. Karsten (2007) concluded, that low water temperatures in Kongsfjorden may contribute to reduced photosynthetic tolerance. He based this hypothesis on the Q_{10}-rule, after which primary metabolism and hence acclimation processes are slowed down under Arctic conditions (Davison 1987). Conversely, warmer temperatures will support the capability of *S. latissima* to counteract variations in salinity. The fact that the number of differentially expressed genes in *S. latissima* cultures was lower at 15 °C at low salinity than at 8 and 0 °C support this hypothesis (Machado Monteiro et al. 2019, Li et al. 2020).

Growth of *S. latissima* is limited in summer, due to N limitation. Enhanced growth of kelp under nutrient-enriched conditions was reported previously (e.g. Conolly & Drew 1985, Gerard 1997, Gao et al. 2016) but could not be confirmed. At higher temperatures (8 and 10 °C), addition of nutrients possibly supported growth, even though the interaction was not significant. The diminishing impact of low temperatures on growth possibly outcompeted additional nutrient supply. The F_v/F_m values endorse this hypothesis. Photosynthetic performances of several seaweeds were positively affected by nutrients (Gerard 1997, Henley & Dunton 1997, Figueroa et al. 2009, Gao et al. 2016). At the same time, low temperatures diminished photosynthetic performance but the interaction of additional nutrients and temper-

ature increase led to higher F_v/F_m values. Our results confirmed that potential nutrient enrichment in summer in combination with enhanced temperatures would support growth and fitness of *S. latissima* sporophytes.

Even though photosynthetic efficiency was affected by temperature, the absolute pigment content did not vary, as reported before (e.g. Andersen et al. 2013, Li et al. 2020, Monteiro et al. 2020), probably because former studies worked with higher amplitudes of the abiotic drivers. Nevertheless, comparable to e.g. Olischläger et al. (2017), Li et al. (2020) and Monteiro et al. (2020), we detected a decreasing de-epoxidation state of the xanthophyll cycle (DPS) with increasing temperatures. We conclude that a lower DPS in Arctic *S. latissima*, indicates a reduced overall stress burden mediated by enhanced temperatures.

An impact of temperature on growth, F_v/F_m and DPS was only detected in the temperature × nutrients experiment. Potentially, the diatom contamination in the 8 and 10 °C treatments of the temperature × salinity experiment contributed to physiological stress for the samples by shading, hence, interfering with the effects of increasing temperature. However, previous treatment with ½ PES during the wound healing and acclimation phase may have reduced any subsequent signal of the nutrient treatments (Fernández et al. 2020).

Comparable to the Arctic endemic kelp *Laminaria solidungula* (Diehl et al. 2020), C content remained the same throughout both experiments. The C:N ratio and N content were also not affected by salinity or by nutrients, or by their respective interactions with temperature. Thus, increased N content at hyposalinity (e.g. Gordillo et al. 2002, Diehl et al. 2020) or in N-treated samples (Gerard 1997) could not be confirmed. A temperature impact was only detected in the temperature × salinity experiment, where samples were constantly kept at the ½ PES. Fernández et al. (2020) showed that enhanced N ameliorated negative impacts of high temperatures in *Macrocystis pyrifera*. Hence, ½ PES treatment which *S. latissima* would not experience in nature could have supported performance with increasing temperatures in our study. Increasing temperatures resulted in decreasing N concentration and consequently in increasing C:N ratios. Temperature is known to induce changes in enzymatic reactions and hence affects nutrient uptake and assimilation (Hurd et al. 2014). Additionally, enzymes represent crucial cellular nitrogen reservoirs. Since enzymatic activities are reduced at colder temperatures, higher concentration of enzymatic proteins are required (Hurd et al. 2014, Bischof et al. 2019). Hence, decreasing N content due to temperature increase in Arctic *S. latissima* represents less N demand by enzyme synthesis which, on the one hand, is based on more effective N uptake and assimilation, and on the other hand on reduced requirement of enzymatic proteins. The C:N ratio in these samples was below 20, indicating sufficient N supply,

while it was higher in all initial samples and in the samples of the temperature × nutrients experiment. C:N ratios above 20 indicate that *S. latissima* is N limited *in situ* and in the temperature × nutrients experiment (Atkinson & Smith 1983). Nutrient depletion most probably counteracted any temperature impacts on N uptake in Arctic *S. latissima*. The lack of N supply in Kongsfjorden during summer could not be compensated within two weeks under potentially enriched nutrient regimes (Gordillo et al. 2006, Zacher et al. 2009, Bischof et al. 2019) but after 14 days of cultivation in ½ PES. Long-term impacts should thus be investigated further.

In contrast to the Arctic endemic *L. solidungula* (Diehl et al. 2020), mannitol did not decrease at low temperatures and the concentrations overall were very high compared to other studies on *S. latissima* (Sharma et al. 2018, Monteiro et al. 2020). Even though the different nutrient treatments had no impact, mannitol concentration decreased at 10 °C in the temperature × nutrient experiment. De novo synthesized mannitol is probably directly converted to the long-term storage product laminarin (Yamaguchi et al. 1966, Graiff et al. 2016, Scheschonk et al. 2019) at higher temperatures. Contrary to other studies (e.g. Karsten 2007, Diehl et al. 2020, Monteiro et al. 2020), we could not detect any impact of salinity variations. Sugar alcohols have been reported as cryoprotectant agents (Elliott et al. 2017). Recently, mannitol was confirmed as to be protective against freezing at 0 °C in *S. latissima* from Roscoff (Monteiro et al. 2020). Li et al. (2020), who worked with a similar experimental set-up as Monteiro et al. (2020), using lab cultures of Arctic *S. latissima*, could not detect any changes in gene expression of mannitol-1-P dehydrogenase at 0 °C or at hyposalinity. Apparently, Arctic *S. latissima* constantly stores high concentrations of mannitol as an acclimation to cold temperatures and regular salinity fluctuations.

Temperature increase had no significant effect on phlorotannin content in *S. latissima*, and neither did nitrate/phosphate enrichment. However, in accordance with previous reports on brown macroalgae, phlorotannin concentrations in *S. latissima* were higher at the control salinity than at hyposalinity (Amsler 2008 and references therein, Kamiya et al. 2010). As hypothesized by Springer et al. (2017), membrane-bound physodes which store phlorotannins might play a role in reinforcing cell walls under osmotic stress, but further investigations for verification are needed. Although in both experiments phlorotannin concentrations at the end differed from the initial concentrations, we can reject the hypothesis that osmotic stress alone induced the variations in phlorotannin content, since all samples were cultivated under the same conditions (control salinity, ½ PES). Phlorotannins do not only act as a feeding deterrent but also as antioxidants in kelp and are affected by different light environments (Amsler 2008

and references therein). Light availability is known to have a greater effect on brown algal phlorotannin content than nutrient availability (Pavia & Toth 2000, Gao et al. 2016). Most likely, cultivation under a constant light regime triggered the phlorotannin accumulation in this study.

Ecological implications for S. latissima *in the future Arctic*

Saccharina latissima is a kelp species with a broad distributional range, which is based on a high degree of polymorphism and plastic physiological responses (Bartsch et al. 2008). Overall, *S. latissima* exhibits wide acclimation capabilities to different environmental variations (e.g. Bischof et al. 1998, Karsten 2007, Nielsen et al. 2016).

Our study was set out to apply realistic amplitudes of change for the environmental variables temperature, salinity and nutrients in a high-Arctic fjord system (Hanelt et al. 2001, Zacher et al. 2009, Meredith et al. 2019), albeit in a rather short-term experiment.

Consistent with its large salinity-tolerance range, *S. latissima* was not harmed by hyposaline conditions in the temperature × salinity experiment. However, constantly high mannitol concentrations may have been applied as an adaptive feature. Apparently, salinity fluctuations in Arctic fjords, which occur due to e.g. sea ice and glaciers melting (Hanelt et al. 2001, Karsten et al. 2003, Karsten 2007), can be efficiently compensated for and accordingly do not harm *S. latissima*.

The temperature × nutrients experiment was designed to mimic potential nutrient enrichment over summer and during marine heatwaves. In agreement with Gordillo et al. (2006), we only determined marginal effects of nutrients on *S. latissima*. Independently from the measured response variables, Arctic *S. latissima* would benefit from nutrient enrichment during the summer, mainly with respect to growth and fitness; as a result, the abundance and biomass of *S. latissima* would increase and consequently also change the habitat for associated organisms, i.e. by outcompeting more sensitive species, such as the endemic kelp *L. solidungula* (Scheschonk et al. 2019, Diehl et al. 2020).

In the Arctic, species with a broad distributional range are more tolerant to environmental changes and have neutral or positive responses to future warming, while species with a narrow distributional range are less tolerant (Kelly et al. 2012, Reich et al. 2015, Sunday et al. 2015). This also applies to kelps in the Arctic (Müller et al. 2009 and references therein). Broadly distributed species will benefit, while endemic and more sensitive species will suffer

from temperature increase (Müller et al. 2009, Iñiguez et al. 2016, Diehl et al. 2020). In Kongsfjorden, *S. latissima* has experienced only little change in biomass during the last 20 yr and a slight reduction in depth extension (Bartsch et al. 2016), but overall a global northward shift is expected (Müller et al. 2009). Here we confirm that *S. latissima* also benefits from short-term temperature increases, i.e. under heatwave events in the Arctic. The potential beneficial impact from nutrient enrichment or the adverse impact from salinity fluctuations will most likely only play a minor role. Hence, we conclude that, due to its high adaptability, *S. latissima* is tolerant to and might even be promoted by environmental changes caused by climate change in the Arctic. Observations of increased *S. latissima* populations made by scientific divers in Kongsfjorden over the last years, support this conclusion (M. Brand pers. comm.). Nevertheless, long-term experiments and the thorough evaluation of the implication of variations in light climate are required for definite conclusions.

Acknowledgements

This study was conducted at the AWIPEV Research Station in Ny-Ålesund, Svalbard. We are grateful to the station staff for support and logistics, and to the Scientific Diving Team (Markus Brand, Laura Eickelmann and Sarina Niedzwiedz) for collecting the samples. Britta Meyer-Schlosser (University of Bremen) assisted with pigment analyses, and Prof. Ulf Karsten and Juliane Müller (University of Rostock) provided expertise and facilities for analyzing mannitol and C:N ratios. This study was further supported by the 2015 – 2016 BiodivERsA COFUND call for research proposals (program MARFOR), with the national funder German Research Foundation (DFG; grant no. VA 105/25-1).

References

Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. *Mar Biol* 140:1087–1095.

Amsler CD (2008) Algal chemical ecology. Amsler CD (ed) Springer-Verlag Berlin Heidelberg.

Andersen GS, Pedersen MF, Nielsen SL (2013) Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima* (Laminariales, Phaeophyceae). *J Phycol* 49:689–700.

Araújo RM, Assis J, Aguillar R, Airoldi L, Bárbara I, Bartsch I, Bekkby T, Christie H, Davoult D, Derrien-Courtel S, Fernandez C, Fredriksen S, Gevaert F, Gundersen H, Le Gal A, Lévêque L, Mieszkowska N, Norderhaug KM, Oliveira P, Puente A, Rico JM, Rinde E, Schubert H, Strain EM, Valero M, Viard F, Sousa-Pinto I (2016) Status, trends and drivers of kelp forests in Europe: An expert assessment. *Biodivers Conserv* 25:1319–1348.

Atkinson MJ, Smith SV (1983) C:N:P ratios of benthic marine plants. *Limnol Oceanogr* 28:568–574.

AWI (Alfred Wegener Institute) (2019) Water temperatures – NyÅlesund/Svalbard – N78°55.200/ E11°54.00. https://dashboard.awi.de/?dashboard=2847 (accessed 11 Sept 2019).

Bartsch I, Paar M, Fredriksen S, Schwanitz M, Daniel C, Hop H, Wiencke C (2016) Changes in kelp forest biomass and depth distribution in Kongsfjorden, Svalbard, between 1996–1998 and 2012–2014 reflect Arctic warming. *Polar Biol* 39:2021–2036.

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J (2008) The genus *Laminaria sensu lato*: Recent insights and developments. *Eur J Phycol* 43:1–86.

Bischof K, Buschbaum C, Fredriksen S, Gordillo FJL, Heinrich S, Jiménez C, Lütz C, Molis M, Roleda MY, Schwanitz M, Wiencke C (2019) Kelps and environmental changes in Kongsfjorden: Stress perception and responses. In: *The ecosystem of Kongsfjorden, Svalbard. Advances in polar ecology*, Vol 2. Hop H, Wiencke C (eds) Springer International, Cham, p 373–422.

Bischof K, Hanelt D, Aguilera J, Karsten U, Vögele B, Sawall T, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. *Mar Biol* 140:1097–1106.

Bischof K, Hanelt D, Tüg H, Karsten U, Brouwer PEM, Wiencke C (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biol* 20:388–395.

Bluhm BA, Gradinger R (2008) Regional variability in food availability for Arctic marine mammals. *Ecol Appl (Suppl):*S77–S96 .

Brown AD, Simpson JR (1972) Water relations of sugar-tolerant yeasts: The role of intracellular polyols. *J Gen Microbiol* 72:589–591.

Colombo-Pallotta MF, García-Mendoza E, Ladah LB (2006) Photosynthetic performance, light absorption, and pigment composition of *Macrocystis pyrifera* (Laminariales, Phaeophyceae) blade from different depths. *J Phycol* 42:1225–1234.

Conolly NJ, Drew EA (1985) Physiology of *Laminaria*: III. Effect of a coastal eutrophication gradient on seasonal patterns of growth and tissue composition in *L. digitata* Lamour. and *L. saccharina* (L.) Lamour. *Mar Ecol* 6:181–195.

Cruces E, Huovinen P, Gómez I (2012) Phlorotannin and antioxidant responses upon short-term exposure to UV radiation and elevated temperature in three south Pacific kelps. *Photochem Photobiol* 88:58–66.

Dalpadado P, Hop H, Rønning J, Pavlov V, Sperfeld E, Buchholz F, Rey A, Wold A (2016) Distribution and abundance of euphausiids and pelagic amphipods in Kongsfjorden, Isfjorden and Rijpfjorden (Svalbard) and changes in their relative importance as key prey in a warming marine ecosystem. *Polar Biol* 39:1765–1784.

Davison IR (1987) Adaptation of photosynthesis in *Laminaria saccharina* (Phaeophyta) to changes in growth temperature. *J Phycol* 23:273–283.

Davison IR, Davison JO (1987) The effect of growth temperature on enzyme activities in the brown alga *Laminaria saccharina*. *Br Phycol J* 22:77–87.

Davison IR, Greene RM, Podolak EJ (1991) Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina*. *Mar Biol* 110:449–454.

Diehl N, Karsten U, Bischof K (2020) Impacts of combined temperature and salinity stress on the endemic Arctic brown seaweed *Laminaria solidungula* J. Agardh. *Polar Biol* 43:647–656.

Dring MJ (2005) Stress resistance and disease resistance in seaweeds: The role of reactive oxygen metabolism. *Adv Bot Res* 43:175–207.

Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K (1996) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of *Laminaria* (Phaeophyta). *Mar Biol* 126:183–191.

Elliott GD, Wang S, Fuller BJ (2017) Cryoprotectants: A review of the actions and applications of cryoprotective solutes that modulate cell recovery from ultra-low temperatures. *Cryobiology* 76:74–91.

Endo H, Suehiro K, Gao X, Agatsuma Y (2017) Interactive effects of elevated summer temperature, nutrient availability, and irradiance on growth and chemical compositions of juvenile kelp, *Eisenia bicyclis*. *Phycol Res* 65:118–126.

Fernandes F, Barbosa M, Oliveira AP, Azevedo IC, Sousa-Pinto I, Valentão P, Andrade PB (2016) The pigments of kelps (Ochrophyta) as part of the flexible response to highly variable marine environments. *J Appl Phycol* 28:3689–3696.

Fernández PA, Gaitán-Espitia JD, Leal PP, Schmid M, Revill AT, Hurd CL (2020) Nitrogen sufficiency enhances thermal tolerance in habitat-forming kelp: Implications for acclimation under thermal stress. *Sci Rep* 10:3186.

Figueroa FL, Israel A, Neori A, Martínez B, Malta E, Ang Jr. P, Inken S, Marquardt R, Korbee N (2009) Effects of nutrient supply on photosynthesis and pigmentation in *Ulva lactuca* (Chlorophyta): Responses to short-term stress. *Aquat Biol* 7:173–183.

Filbee-Dexter K, Wernberg T, Fredriksen S, Norderhaug KM, Pedersen MF (2019) Arctic kelp forests: Diversity, resilience and future. *Glob Planet Change* 172:1–14.

Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helg. Meeresunt* 34:15–29.

Fredersdorf J, Müller R, Becker S, Wiencke C, Bischof K (2009) Interactive effects of radiation, temperature and salinity on different life history stages of the Arctic kelp *Alaria esculenta* (Phaeophyceae). *Oecologia* 160:483–492.

Gao X, Endo H, Nagaki M, Agatsuma Y (2016) Growth and survival of juvenile sporophytes of the kelp *Ecklonia cava* in response to different nitrogen and temperature regimes. *Fish Sci* 82:623–629.

Gerard VA (1997) The role of nitrogen nutrition in high-temperature tolerance of the kelp, *Laminaria saccharina* (Chromophyta). *J Phycol* 33:800–810.

Gerard VA, DuBois K, Greene R (1987) Growth responses of two *Laminaria saccharina* populations to environmental variation. *Hydrobiologia* 151-152:229–232.

Gordillo FJL, Aguilera J, Jiménez C (2006) The response of nutrient assimilation and biochemical composition of Arctic seaweeds to a nutrient input in summer. *J Exp Bot* 57:2661–2671.

Gordillo FJL, Dring MJ, Savidge G (2002) Nitrate and phosphate uptake characteristics of three species of brown algae cultured at low salinity. *Mar Ecol Prog Ser* 234:111–118.

Graiff A, Bartsch I, Ruth W, Wahl M, Karsten U (2015) Season exerts differential effects of ocean acidification and warming on growth and carbon metabolism of the seaweed *Fucus vesiculosus* in the western Baltic Sea. *Front Mar Sci* 2:112.

Graiff A, Ruth W, Kragl U, Karsten U (2016) Chemical characterization and quantification of the brown algal storage compound laminarin – A new methodological approach. *J Appl Phycol* 28:533–543.

Hanelt D, Tüg H, Bischof K, Groß C, Lippert H, Sawall T, Wiencke C (2001) Light regime in an Arctic fjord: A study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Mar Biol* 138:649–658.

Henley WJ, Dunton KH (1997) Effects of nitrogen supply and continuous darkness on growth and photosynthesis of the arctic kelp *Laminaria solidungula*. *Limnol Oceanogr* 42:209–216.

Hobday AJ, Alexander LV, Perkins SE, Smale DA, Straub SC, Oliver ECJ, Benthuysen JA, Burrows MT, Donat MG, Feng M, Holbrook NJ, Moore PJ, Scannell HA, Sen Gupta A, Wernberg T (2016) A hierarchical approach to defining marine heatwaves. *Prog Oceanogr* 141:227–238.

Hop H, Kovaltchouk NA, Wiencke C (2016) Distribution of macroalgae in Kongsfjorden, Svalbard. *Polar Biol* 39:2037–2051.

Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed ecology and physiology, Second Edi. Cambridge University Press, Cambridge.

Iñiguez C, Carmona R, Lorenzo MR, Niell FX, Wiencke C, Gordillo FJL (2016) Increased temperature, rather than elevated CO_2, modulates the carbon assimilation of the Arctic kelps *Saccharina latissima* and *Laminaria solidungula*. *Mar Biol* 163:248.

Iwamoto K, Shiraiwa Y (2005) Salt-regulated mannitol metabolism in algae. *Mar Biotechnol* 7:407–415.

Kamiya M, Nishio T, Yokoyama A, Yatsuya K, Nishigaki T, Yoshikawa S, Ohki K (2010) Seasonal variation of phlorotannin in sargassacean species from the coast of the Sea of Japan. *Phycol Res* 58:53–61.

Karsten U (2007) Salinity tolerance of Arctic kelps from Spitsbergen. *Phycol Res* 55:257–262.

Karsten U (2012) Seaweed acclimation to salinity and desiccation stress. In: *Seaweed Biology – Novel insights into ecophysiology, ecology and utilization*. Wiencke C, Bischof K (eds) Springer-Verlag, Berlin, Heidelberg p 87–107.

Karsten U, Dummermuth A, Hoyer K, Wiencke C (2003) Interactive effects of ultraviolet radiation and salinity on the ecophysiology of two Arctic red algae from shallow waters. *Polar Biol* 26:249–258.

Karsten U, Thomas DN, Weykam G, Daniel C, Kirst GO (1991) A simple and rapid method for extraction and separation of low molecular weight carbohydrates from macroalgae using high-performance liquid chromatography, II. Intracellular inorganic ions and organic compounds. *Plant Physiol Biochem* 29:373–378.

Kelly MW, Sanford E, Grosberg RK (2012) Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proc R Soc B* 279:349–356.

Kirst GO (1989) Salinity tolerance of eukaryotic marine algae. *Annu Rev Plant Physiol Plant Mol Biol* 40:21–53.

Koch K, Thiel M, Tellier F, Hagen W, Graeve M, Tala F, Laeseke P, Bischof K (2015) Species separation within the *Lessonia nigrescens* complex (Phaeophyceae, Laminariales) is mirrored by ecophysiological traits. *Bot Mar* 58:81–92.

Korb RE, Gerard VA (2000a) Effects of concurrent low temperature and low nitrogen supply on polar and temperate seaweeds. *Mar Ecol Prog Ser* 198:73–82.

Korb RE, Gerard VA (2000b) Nitrogen assimilation characteristics of polar seaweeds from differing nutrient environments. *Mar Ecol Prog Ser* 198:83–92.

Krause-Jensen D, Duarte CM (2014) Expansion of vegetated coastal ecosystems in the future Arctic. *Front Mar Sci* 1:77.

Lane CE, Mayes C, Druehl LD, Saunders GW (2006) A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. *J Phycol* 42:493–512.

Li H, Monteiro C, Heinrich S, Bartsch I, Valentin K, Harms L, Glöckner G, Corre E, Bischof K (2020) Responses of the kelp *Saccharina latissima* (Phaeophyceae) to the warming Arctic: From physiology to transcriptomics. *Physiol Plant* 168:5–26.

Mabin CJT, Gribben PE, Fischer A, Wright JT (2013) Variation in the morphology, reproduction and development of the habitat-forming kelp *Ecklonia radiata* with changing temperature and nutrients. *Mar Ecol Prog Ser* 483:117–131.

Machado Monteiro CM, Li H, Bischof K, Bartsch I and others (2019) Is geographical variation driving the transcriptomic responses to multiple stressors in the kelp *Saccharina latissima*? *BMC Plant Biol* 19:513.

Mandal SK, Ajay G, Monisha N, Malarvizhi J, Temkar G, Mantri VA (2015) Differential response of varying temperature and salinity regimes on nutrient uptake of drifting fragments of *Kappaphycus alvarezii*: Implication on survival and growth. *J Appl Phycol* 27:1571–1581.

Meredith M, Sommerkorn M, Cassotta S, Derksen S, Ekaykin A, Hollowed A, Kofinas G, Mackintosh A, Melbourne-Thomas J, Muelbert MMC, Ottersen G, Pritchard H, Schuur EAG (2019) Polar regions. In: *IPCC Special report on the ocean and cryosphere in a changing climate*. Pörtner H-O, Roberts DC, Masson-Delmotte V, Zhai P and others (eds) p 203–320.

Monteiro C, Li J, Diehl N, Collén J, Heinrich S, Bischof K, Bartsch I (2020) Modulation of physiological performance by temperature and salinity in the sugar kelp *Saccharina latissima*. *Phycol Res* online.

Moy FE, Christie H (2012) Large-scale shift from sugar kelp (*Saccharina latissima*) to ephemeral algae along the south and west coast of Norway. *Mar Biol Res* 8:309–321.

Müller R, Laepple T, Bartsch I, Wiencke C (2009) Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Bot Mar* 52:617–638.

Nepper-Davidsen J, Andersen DT, Pedersen MF (2019) Exposure to simulated heat wave scenarios causes long-term reductions in performance in *Saccharina latissima*. *Mar Ecol Prog Ser* 630:25–39.

Nielsen MM, Manns D, D'Este M, Krause-Jensen D, Rasmussen MB, Larsen MM, Alvarado-Morales M, Angelidaki I, Bruhn A (2016) Variation in biochemical composition of *Saccharina latissima* and *Laminaria digitata* along an estuarine salinity gradient in inner Danish waters. *Algal Res* 13:235–245.

Olischläger M, Iñiguez C, Koch K, Wiencke C, Gordillo FJL (2017) Increased pCO_2 and temperature reveal ecotypic differences in growth and photosynthetic performance of temperate and Arctic populations of *Saccharina latissima*. *Planta* 245:119–136.

Pavia H, Toth G (2000) Influence of light and nitrogen on the phlorotannin content of the brown seaweed *Ascophyllum nodosum* and *Fucus vesiculosus*. *Hydrobiologia* 440:299–305.

Pfister CA, Van Alstyne KL (2003) An experimental assessment of the effects of nutrient enhancement on the intertidal kelp *Hedophyllum sessile* (Laminarales, Phaeophyceae). *J Phycol* 39:285–290.

Provasoli L (1968) Media and prospects for the cultivation of marine algae. In: *Cultures and collections of algae. Proceedings of the Japanese Conference Hakone*. Watanabe A, Hattori A (eds) Japanese Society of Plant Physiology. Tokyo, p 63–75.

Rautenberger R, Bischof K (2006) Impact of temperature on UV-susceptibility of two *Ulva* (Chlorophyta) species from Antarctic and Subantarctic regions. *Polar Biol* 29:988–996.

Reich PB, Sendall KM, Rice K, Rich RL, Stefanski A, Hobbie SE, Montgomery RA (2015) Geographic range predicts photosynthetic and growth response to warming in co-occurring tree species. *Nat Clim Change* 5:148–152.

Rokkan Iversen K, Seuthe L (2011) Seasonal microbial processes in a high-latitude fjord (Kongsfjorden, Svalbard): I. Heterotrophic bacteria, picoplankton and nanoflagellates. *Polar Biol* 34:731–749.

Scheschonk L, Becker S, Hehemann JH, Diehl N, Karsten U, Bischof K (2019) Arctic kelp eco-physiology during the polar night in the face of global warming: A crucial role for laminarin. *Mar Ecol Prog Ser* 611:59–74.

Sharma S, Neves L, Funderud J, Mydland LT, Øverland M, Horn SJ (2018) Seasonal and depth variations in the chemical composition of cultivated *Saccharina latissima*. *Algal Res* 32:107–112.

Simonson EJ, Scheibling RE, Metaxas A (2015) Kelp in hot water: I. Warming seawater temperature induces weakening and loss of kelp tissue. *Mar Ecol Prog Ser* 537:89–104.

Smale DA, Wernberg T, Oliver ECJ, Thomsen M, Harvey BP, Straub SC, Burrows MT, Alexander L V., Benthuysen JA, Donat MG, Feng M, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell HA, Sen Gupta A, Payne BL, Moore PJ (2019) Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nat Clim Change* 9:306–312.

Springer K, Lütz C, Lütz-Meindl U, Wendt A, Bischof K (2017) Hyposaline conditions affect UV susceptibility in the Arctic kelp *Alaria esculenta* (Phaeophyceae). *Phycologia* 56:675–685.

Sunday JM, Pecl GT, Frusher S, Hobday AJ, Hill N, Holbrook NJ, Edgar GJ, Stuart-Smith R, Barrett N, Wernberg T, Watson RA, Smale DA, Fulton EA, Slawinski D, Feng M, Radford BT, Thompson PA, Bates AE (2015) Species traits and climate velocity explain geographic range shifts in an ocean-warming hotspot. *Ecol Lett* 18:944–953.

Sundfjord A, Albretsen J, Kasajima Y, Skogseth R, Nuth C, Skarðhamar J, Cottier F, Nilsen F, Asplin L, Gerland S, Torsvik T (2017) Effects of glacier runoff and wind on surface layer dynamics and Atlantic water exchange in Kongsfjorden, Svalbard; a model study. *Estuar Coast Shelf Sci* 187:260–272.

Svendsen H, Beszczynska-Møller A, Hagen JO, Levauconnier B, Vigdis T, Gerland S, Børre Ørbæk J, Bischof K, Papucci C, Zajaczkowski M, Azzolini R, Bruland O, Wiencke C, Winther J-G, Dallmann W (2002) The physical environment of Kongsfjorden-Krossfjorden, an Arctic fjord system in Svalbard. *Polar Res* 21:133–166.

Tatewaki M (1966) Formation of a crustaceous sporophyte with unilocular sporangia in *Scytosiphon lomentaria*. *Phycologia* 6:62–66.

tom Dieck (Bartsch) I (1992) North Pacific and North Atlantic digitate *Laminaria* species (Phaeophyta): Hybridization experiments and temperature responses. *Phycologia* 31:147–163.

Verardo DJ, Froelich PN, McIntyre A (1990) Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 Analyzer. *Deep Sea Res Part A Oceanogr Res Pap* 37:157–165.

Wiencke C, Bischof K (2012) Seaweed Biology – Novel insights into ecophysiology, ecology and utilization. Springer, Heidelberg.

Wiencke C, Clayton MN, Gómez I, Iken K, Lüder UH, Amsler CD, Karsten U, Hanelt D, Bischof K, Dunton K (2007) Life strategy, ecophysiology and ecology of seaweeds in polar waters. *Rev Environ Sci Biotechnol* 6:95-126.

Wright SW, Jeffrey SW, Mantoura RFC, Llewelly CA, Bjørnland T, Repeta D, Welschmeyer N (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Mar Ecol Prog Ser* 77:183–196.

Yamaguchi T, Ikawa T, Nisizawa K (1966) Incorporation of radioactive carbon from $H^{14}CO_3^-$ into sugar constituents by a brown alga, *Eisenia bicyclis*, during photosynthesis and its fate in the dark. *Plant Cell Physiol* 7:217–229.

Zacher K, Rautenberger R, Hanelt D, Wulff A, Wiencke C (2009) The abiotic environment of polar marine benthic algae. *Bot Mar* 52:483–490.

Supplement 1

Statistics

Temperature × salinity experiment

Table S4.1 Results of the repeated two-way ANOVA for effects of temperature and salinity on the increase of size (% of initial) of *Saccharina latissima* from Spitsbergen measured on t_6, t_8, t_{10}, t_{12}, t_{14} (time). Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*F* value	*p* value
size %[a]	Time	4	97.469	< 0.001*
	Temperature	3	5.354	0.0016*
	Salinity	1	5.593	0.0193*
	Temperature × Salinity	3	15.257	< 0.001*

[a] $\log_{10}$ transformation

Table S4.2 Results of the repeated two-way ANOVA for effects of temperature and salinity on the photosynthetic efficiency F_v/F_m (% of initial) of *Saccharina latissima* from Spitsbergen measured on t_6, t_8, t_{10}, t_{12}, t_{14} (time). Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*F* value	*p* value
F_v/F_m %[a]	Time	4	5.136	< 0.001*
	Temperature	3	0.732	0.53425
	Salinity	1	5.136	0.44404
	Temperature × Salinity	3	0.339	0.79708

[a] ranked data-set (transformation)

Table S4.3 Results of the generalized linear model (GLM) for effects of temperature and salinity compared to initial measurements on chlorophyll *a* (Chl *a*), accessory pigment pool (Acc), xanthophyll pool (VAZ = violaxanthin, antheraxanthin, zeaxanthin), accessory pigments : chlorophyll *a* ratio (Acc:Chl*a*) and the de-epoxidation state of the xanthophyll cycle (DPS) of *Saccharina latissima* from Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	χ^2 value	*p* value
	Within-subjects effects + initial			
Chl *a*	Temperature	3	8.8685	0.0311*
	Salinity	1	1.9622	0.1613
	Temperature × Salinity	3	1.5917	0.6613
Acc	Temperature	3	7.0210	0.0712
	Salinity	1	1.7522	0.1856
	Temperature × Salinity	3	2.3428	0.5044
VAZ	Temperature	3	3.8095	0.2828
	Salinity	1	3.5260	0.0604
	Temperature × Salinity	3	5.0887	0.1654
Acc:Chl*a*[a]	Temperature	3	13.8878	0.0031*
	Salinity	1	0.8609	0.3535
	Temperature × Salinity	3	2.4050	0.4927
DPS	Temperature	3	3.0970	0.3769
	Salinity	1	1.4584	0.2272
	Temperature × Salinity	3	2.2026	0.5314

[a] ranked data-set (transformation)

Table S4.4 Results of the generalized linear model (GLM) and one-way ANOVA for effects of temperature and salinity compared to initial measurements on the C:N ratio, total C and total N content of *Saccharina latissima* from Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects + initial				
C:N	Temperature	ANOVA	4	13.00	< 0.001*
	Salinity	ANOVA	2	13.88	< 0.001*
	Within-subjects effects + initial				
C:N	Temperature	GLM	3	15.3288	0.0016*
	Salinity		1	1.3186	0.2509
	Temperature × Salinity		3	2.5378	0.4685
	Between-subjects effects + initial				
Total C	Temperature	ANOVA	4	0.987	0.429
	Salinity	ANOVA	2	1.637	0.210
	Within-subjects effects + initial				
Total C	Temperature	GLM	3	0.74839	0.8618
	Salinity		1	0.03853	0.8444
	Temperature × Salinity		3	0.64861	0.8852
	Between-subjects effects + initial				
Total N	Temperature	ANOVA	4	6.325	0.0047*
	Salinity	ANOVA	2	18.86	< 0.001*
	Within-subjects effects + initial				
Total N	Temperature	GLM	3	49.449	< 0.001*
	Salinity		1	2.894	0.0889
	Temperature × Salinity		3	1.876	0.5985

Table S4.5 Results of the one-way ANOVA or non-parametric Kruskal-Wallis test for effects of temperature and salinity compared to initial measurements on mannitol of *Saccharina latissima* from Spitsbergen. Effects were also tested independently by temperature and salinity. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects + initial				
Mannitol	Temperature	Kruskal-Wallis	4	24.496	< 0.001* [x]
	Salinity	Kruskal-Wallis	2	21.285	< 0.001* [x]
	Within-subjects effects + initial				
Mannitol_4°C	Salinity	Kruskal-Wallis	2	8.985	0.011* [x]
Mannitol_6°C	Salinity	ANOVA	2	7.222	0.006*
Mannitol_8°C	Salinity	ANOVA	2	2.574	0.109
Mannitol_10°C	Salinity	ANOVA	2	8.272	0.004*
Mannitol_low	Temperature	ANOVA	4	6.051	< 0.001 *
Mannitol_control	Temperature	ANOVA	4	2.309	0.086

[x] Dunn-Bonferroni's test: reject H_0 if $p \leq$ alpha/2, alpha = 0.05

Table S4.6 Results of the generalized linear model (GLM) or one-way ANOVA for effects of temperature and salinity compared to initial measurements on phlorotannins of *Saccharina latissima* from Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects + initial				
Phlorotannins[a]	Temperature	ANOVA	4	9.46	< 0.001*
	Salinity	ANOVA	2	37.03	< 0.001*
	Within-subjects effects + initial				
Phlorotannins[a]	Temperature	GLM	3	4.0690	0.2541
	Salinity		1	30.2870	< 0.001*
	Temperature × Salinity		4	3.8938	0.2732

[a] $\log_{10}$ transformation

Temperature × nutrients experiment

Table S4.7 Results of the repeated two-way ANOVA for effects of temperature and nutrients on the increase of size (% of initial) of *Saccharina latissima* from Spitsbergen measured on t_6, t_8, t_{10}, t_{12}, t_{14} (time). Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*F* value	*p* value
size %[a]	Time	4	61.678	< 0.001*
	Temperature	3	17.340	< 0.001*
	Nutrients	1	2.705	0.102
	Temperature × Nutrients	3	0.230	0.875

[a] $\log_{10}$ transformation

Table S4.8 Results of the repeated two-way ANOVA for effects of temperature and nutrients on the photosynthetic efficiency F_v/F_m (% of initial) of *Saccharina latissima* from Spitsbergen measured on t_6, t_8, t_{10}, t_{12}, t_{14} (time). Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*F* value	*p* value
F_v/F_m %[a]	Time	4	5.457	< 0.001*
	Temperature	3	43.486	< 0.001*
	Nutrients	1	0.760	0.38473
	Temperature × Nutrients	3	3.000	0.03255*

[a] $\log_{10}$ transformation

Table S4.9 Results of the generalized linear model (GLM) for effects of temperature and nutrients compared to initial measurements on chlorophyll *a* (Chl *a*), accessory pigment pool (Acc), xanthophyll pool (VAZ = violaxanthin, antheraxanthin, zeaxanthin), accessory pigments : chlorophyll *a* ratio (Acc:Chl*a*) and the de-epoxidation state of the xanthophyll cycle (DPS) of *Saccharina latissima* from Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	χ^2 value	*p* value
	Within-subjects effects + initial			
Chl *a*	Temperature	3	0.3954	0.9412
	Nutrients	1	0.9617	0.3268
	Temperature × Nutrients	3	3.5522	0.3141
Acc	Temperature	3	0.2557	0.9681
	Nutrients	1	1.9907	0.1583
	Temperature × Nutrients	3	3.6266	0.3047
VAZ	Temperature	3	1.5933	0.6609
	Nutrients	1	0.2625	0.6084
	Temperature × Nutrients	3	4.4743	0.2146
Acc:Chl*a*	Temperature	3	1.9796	0.5767
	Nutrients	1	2.8690	0.0903
	Temperature × Nutrients	3	3.4242	0.3307
DPS[a]	Temperature	3	15.7690	0.0013*
	Nutrients	1	0.15322	0.6955
	Temperature × Nutrients	3	4.0870	0.2522

[a] reciprocal (transformation)

Table S4.10 Results of the generalized linear model (GLM) and one-way ANOVA or non-parametric Kruskal-Wallis test for effects of temperature and nutrients compared to initial measurements on the C:N ratio, total C and total N content of *Saccharina latissima* from Spitsbergen. Effects in C:N ratio were also tested independently by temperature and nutrients. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects + initial				
C:N	Temperature	Kruskal-Wallis	4	9.4872	0.0501
	Nutrients	Kruskal-Wallis	2	2.4036	0.3007
	Within-subjects effects + initial				
C:N_4°C	Nutrients	Kruskal-Wallis	2	1.1429	0.5647
C:N _6°C	Nutrients	Kruskal-Wallis	2	1.1429	0.5647
C:N _8°C	Nutrients	Kruskal-Wallis	2	0.8571	0.6514
C:N _10°C	Nutrients	Kruskal-Wallis	2	0.2857	0.8669
C:N _SW	Temperature	Kruskal-Wallis	4	2.4	0.6626
C:N _SW+N+P	Temperature	Kruskal-Wallis	4	4.8	0.3084
	Between-subjects effects + initial				
Total C	Temperature	ANOVA	4	1.613	0.1980
	Nutrients	ANOVA	2	0.529	0.5940
	Within-subjects effects + initial				
Total C	Temperature	GLM	3	8.0698	0.0446*
	Nutrients		1	1.7119	0.1907
	Temperature × Nutrients		3	8.2049	0.0419*
	Between-subjects effects + initial				
Total N	Temperature	ANOVA	4	2.677	0.0516
	Nutrients	ANOVA	2	1.049	0.3630
	Within-subjects effects + initial				
Total N	Temperature	GLM	3	3.2482	0.0562
	Nutrients		1	1.1118	0.2917
	Temperature × Nutrients		3	2.6964	0.4409

Table S4.11 Results of the one-way ANOVA or non-parametric Kruskal-Wallis test for effects of temperature and nutrients compared to initial measurements on mannitol of *Saccharina latissima* from Spitsbergen. Effects were also tested independently by temperature and nutrients. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	*F* value / χ^2 value	*p* value
	Between-subjects effects + initial				
Mannitol[a]	Temperature	Kruskal-Wallis	4	30.556	< 0.001* [x]
	Nutrients	Kruskal-Wallis	2	0.071	0.9652
	Within-subjects effects + initial				
Mannitol_4°C	Nutrients	ANOVA	2	0.219	0.806
Mannitol_6°C[a]	Nutrients	ANOVA	2	1.515	0.252
Mannitol_8°C	Nutrients	ANOVA	2	3.154	0.0829
Mannitol_10°C	Nutrients	Kruskal-Wallis	2	4.456	0.1077
Mannitol_SW	Temperature	ANOVA	4	0.649	0.633
Mannitol_SW+N+P	Temperature	ANOVA	4	7.733	< 0.001*

[a] reciprocal (transformation)
[x] Dunn-Bonferroni's test: reject H_0 if $p \leq$ alpha/2, alpha = 0.05

Table S4.12 Results of the generalized linear model (GLM) or non-parametric Kruskal-Wallis test for effects of temperature and nutrients compared to initial measurements on phlorotannins of *Saccharina latissima* from Spitsbergen. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor		*df*	χ^2 value	*p* value
	Between-subjects effects + initial				
Phlorotannins[a]	Temperature	Kruskal-Wallis	4	19.527	< 0.001* [x]
	Nutrients	Kruskal-Wallis	2	15.248	< 0.001* [x]
	Within-subjects effects + initial				
Phlorotannins[b]	Temperature	GLM	3	10.3303	0.01596*
	Nutrients		1	4.5437	0.03304*
	Temperature × Nutrients		4	10.2102	0.01686*

[a] $\log_{10}$ transformation [b] ranked data-set (transformation)
[x] Dunn-Bonferroni's test: reject H_0 if $p \leq$ alpha/2, alpha = 0.05

Supplement 2

Physiological and biochemical extra data

Table S4.13: Maximum quantum yield of photosystem II (F_v/F_m) of *Saccharina latissima* as absolute values on t_0, t_6 and t_{14} during the two-factor treatment at four temperatures (4, 6, 8, 10 °C) and **a:** salinity treatments (low, control) or **b:** nutrient treatments (SW: seawater, SW+N+P: seawater + nitrate + phosphate). t_0 was the start of the experiment (initial), t_6 was after the temperature acclimation and t_{14} at the end of the experiment. Values are means ± SD (n = 4). [x] marks diatom contamination.

	Treatments		F_v/F_m		
			t_0	t_6	t_{14}
(a) Temperature × Salinity	4 °C	low	0.697 (± 0.012)	0.697 (± 0.012)	0.708 (± 0.009)
		control	0.692 (± 0.007)	0.692 (± 0.007)	0.685 (± 0.010)
	6 °C	low	0.682 (± 0.013)	0.682 (± 0.013)	0.684 (± 0.009)
		control	0.688 (± 0.007)	0.688 (± 0.007)	0.694 (± 0.008)
	8 °C	low[x]	0.679 (± 0.008)	0.679 (± 0.008)	0.669 (± 0.003)
		control[x]	0.682 (± 0.002)	0.682 (± 0.002)	0.679 (± 0.006)
	10 °C	low[x]	0.691 (± 0.007)	0.691 (± 0.007)	0.689 (± 0.004)
		control[x]	0.671 (± 0.015)	0.671 (± 0.015)	0.683 (± 0.004)
(b) Temperature × Nutrients	4 °C	SW	0.662 (± 0.011)	0.662 (± 0.011)	0.673 (± 0.008)
		SW+N+P	0.654 (± 0.009)	0.654 (± 0.009)	0.653 (± 0.044)
	6 °C	SW	0.671 (± 0.003)	0.671 (± 0.003)	0.657 (± 0.020)
		SW+N+P	0.676 (± 0.010)	0.676 (± 0.010)	0.680 (± 0.008)
	8 °C	SW	0.680 (± 0.009)	0.680 (± 0.009)	0.684 (± 0.011)
		SW+N+P	0.664 (± 0.011)	0.664 (± 0.011)	0.679 (± 0.011)
	10 °C	SW	0.679 (± 0.013)	0.679 (± 0.013)	0.668 (± 0.018)
		SW+N+P	0.672 (± 0.011)	0.672 (± 0.011)	0.687 (± 0.016)

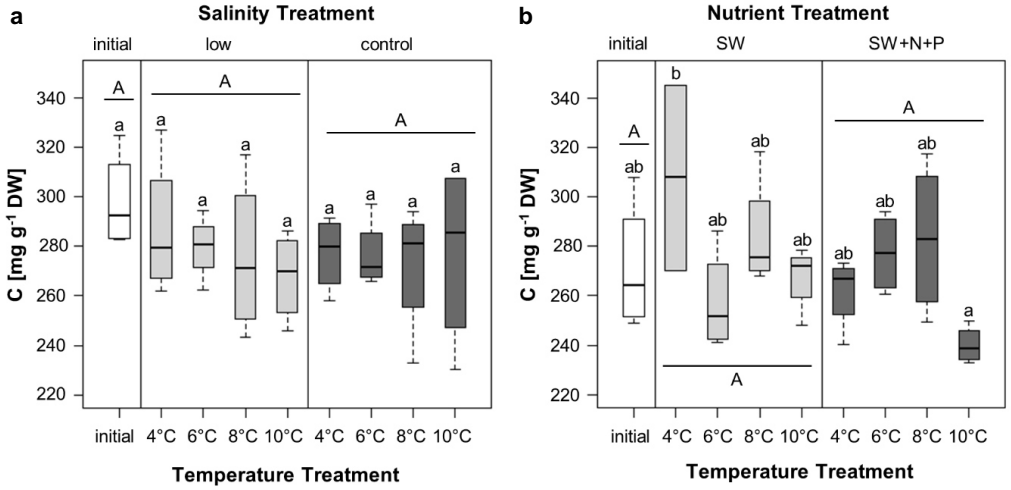


Fig. S4.1: Total C content in *Saccharina latissima* after one week of two-factor treatment at four temperatures (4, 6, 8, 10 °C) and **a:** salinity treatments (low, control) or **b:** nutrient treatments (SW: seawater , SW+N+P: seawater + nitrate + phosphate). The boxplots represent the median (50th percentile), the interquartile range (25th to the 75th percentile) and the minimum and maximum values of the data-sets. Significant differences are marked with different letters; lowercase letters mark within-subjects effects and uppercase letters between-subjects effects (n = 4; two-way ANOVA with post hoc Tukey's test; $p < 0.05$).

5. Publication IV:

Exploring intraspecific variability – Biochemical and morphological characteristics of the kelp *Saccharina latissima* along latitudinal and salinity gradients in Europe

Nora Diehl, Niko Steiner, Kai Bischof, Svenja Heesch

Author to be included before submission: Ulf Karsten

in preparation

Title: Exploring intraspecific variability – Biochemical and morphological characteristics of the kelp *Saccharina latissima* along latitudinal and salinity gradients in Europe

Authors: Nora Diehl, Niko Steiner, Kai Bischof, Svenja Heesch

Abstract

Broadly distributed seaweeds, such as the boreal-temperate kelp species *Saccharina latissima*, contain a multitude of biochemical compounds helping to adjust to changes in their environment, such as temperature and salinity. In Europe, *S. latissima* is found along Atlantic coasts from Spitsbergen to Portugal, including the Baltic Sea. Along this extensive gradient the species exhibits great morphological plasticity. By statistically linking morphological, biochemical, genetic data to geographic information, we aimed to obtain insights into the site-specific adaptive features of *S. latissima*. Hence, we investigated the morphological and biochemical traits of sporophytes from 16 different locations across its entire distributional range in relation to local abiotic conditions (sea surface temperature, salinity, sampling depth). While frond length and width, mannitol, C:N and phlorotannins showed strong intraspecific variability dependent on local abiotic drivers, we did not observe distinct genetic clusters according to their geographical origin and phenotypical features. Also, populations from the Baltic Sea were not genetically distinguishable from fully marine populations, even though sporophyte size strongly decreased along the salinity gradient in the Baltic Sea. Despite the apparent impact of local abiotic factors on specimens' morphology, we could not determine habitat-specific signatures in the biochemical phenotypes of *S. latissima*. Our findings provide a base for studying separation processes and conservation ecology.

Introduction

Kelps, i.e. brown seaweeds belonging to the order Laminariales, are important primary producers and ecosystem engineers in coastal marine ecosystems around the globe (Dayton 1985, Bartsch et al. 2008). *Saccharina latissima* (L.) Lane et al. is a boreal-temperate kelp species occurring from the Arctic down to latitudes of ~ 40°N (Araújo et al. 2016). It grows on rocky shores and prefers sheltered conditions (Lüning 1990) with a depth distribution from the upper subtidal to depths of 15 – 30 m (Wiencke et al. 2004, Pehlke & Bartsch 2008, Bekkby & Moy 2011, Bischof et al. 2019). The species generally tolerates large variations in environmental conditions (e.g. Bischof et al. 1998, Karsten 2007, Nielsen et al. 2016a). Its optimum growth activity ranges between 10 and 15 °C (Bolton & Lüning 1982), but the species can survive temperatures between 0 and 23 °C (Fortes & Lüning 1980, Bolton & Lüning 1982, Lüning 1984). Recently, Diehl et al. (submitted) revealed that field sporophytes can even survive temperatures up to 25 °C for shorter periods. Besides temperature, other abiotic drivers, such as salinity, light and nutrient availability, affect the distribution of *S. latissima* (Lüning 1990). Regarding salinity, optimum growth occurs between an absolute salinity (S_A) of 23 and 31, with strong reductions in growth below S_A 16 and high mortality at S_A < 8 (Gerard et al. 1987, Karsten 2007). This trait allows *S. latissima* to locally establish also in the Baltic Sea, which is characterized by a strong salinity gradient from west to east (Viktorsson 2017). Indeed, stable populations of *S. latissima* are recorded from the Western Baltic Sea (Araújo et al. 2016), although from the Skagerrak eastwards, individuals descend to greater depths (Nielsen et al. 2016a). In the south-west, where salinity is around S_A 10, *S. latissima* is only rarely found (Kautsky & Kautsky 2000, Araújo et al. 2016).

Like other seaweeds, *S. latissima* produces a multitude of biochemical compounds involved in defense, signaling and acclimation processes (Amsler 2008). Mannitol, as 'compatible solute', maintains the intracellular functions under osmotic stress (Karsten 2012), while phlorotannins are multifunctional and, for instance, act as antioxidants under high light conditions (Amsler 2008). The C:N ratio reflects the nutrient uptake and assimilation, concomitant with protein concentrations and nutrient supply (Hurd et al. 2014). The specific chemical profile of a specimen mirrors the environmental conditions at the original growth site (Martins et al. 2014, Monteiro et al. 2020) and, hence, reflects local adaptation on large geographical scales. Along European shores, populations of *S. latissima* show pronounced differentiation, with genetically separated populations observed even within small geographical distances (Guzinski et al. 2016, Nielsen et al. 2016b, Luttikhuizen et al. 2018). Guzinski et al. (2020)

revealed the existence of a high degree of inter-population differentiation but did not find significant variation in genetic diversity with latitude. Accordingly, intraspecific diversity of *S. latissima* could represent an intermediate stage of an ongoing speciation process enabled by high phenotypic plasticity.

In this study, we correlated morphological features and biochemical composition of *S. latissima* and related abiotic conditions at 16 sampling sites across its entire distributional range in Europe. We aimed to reveal the respective variability as prerequisite for habitat-specific signatures in the species and hypothesized that the biochemical phenotypes of *S. latissima* reflect their geographical origin. Bolton (2010) stated that "combined studies of the morphology, molecular systematics, and ecology of the speciation process will be important for our future understanding of kelp evolution" (Bolton 2010, p. 275f). Hence, while genetically confirming species identifications in our samples, we additionally tested whether haplotypes allowed for the detection of distinct genetic clusters according to their geographical origin and biochemical profiles.

Material and methods

Sampling

Meristematic parts from adult sporophytes of *Saccharina latissima* were collected from 16 different locations along the European Atlantic coast and the Baltic Sea in the summer periods of 2018, 2019 and 2020 (**Fig. 5.1a**, **Table 5.1**). The map (copyrights: EuroGeographics for the administrative boundaries) was generated using QGIS 3.8.2-Zanzibar software (QGIS Development Team 2019). The samples were gently dried depending on respective logistic facilities, either in drying cabinets at 40 – 45 °C or in silica gel (Stévant et al. 2018).

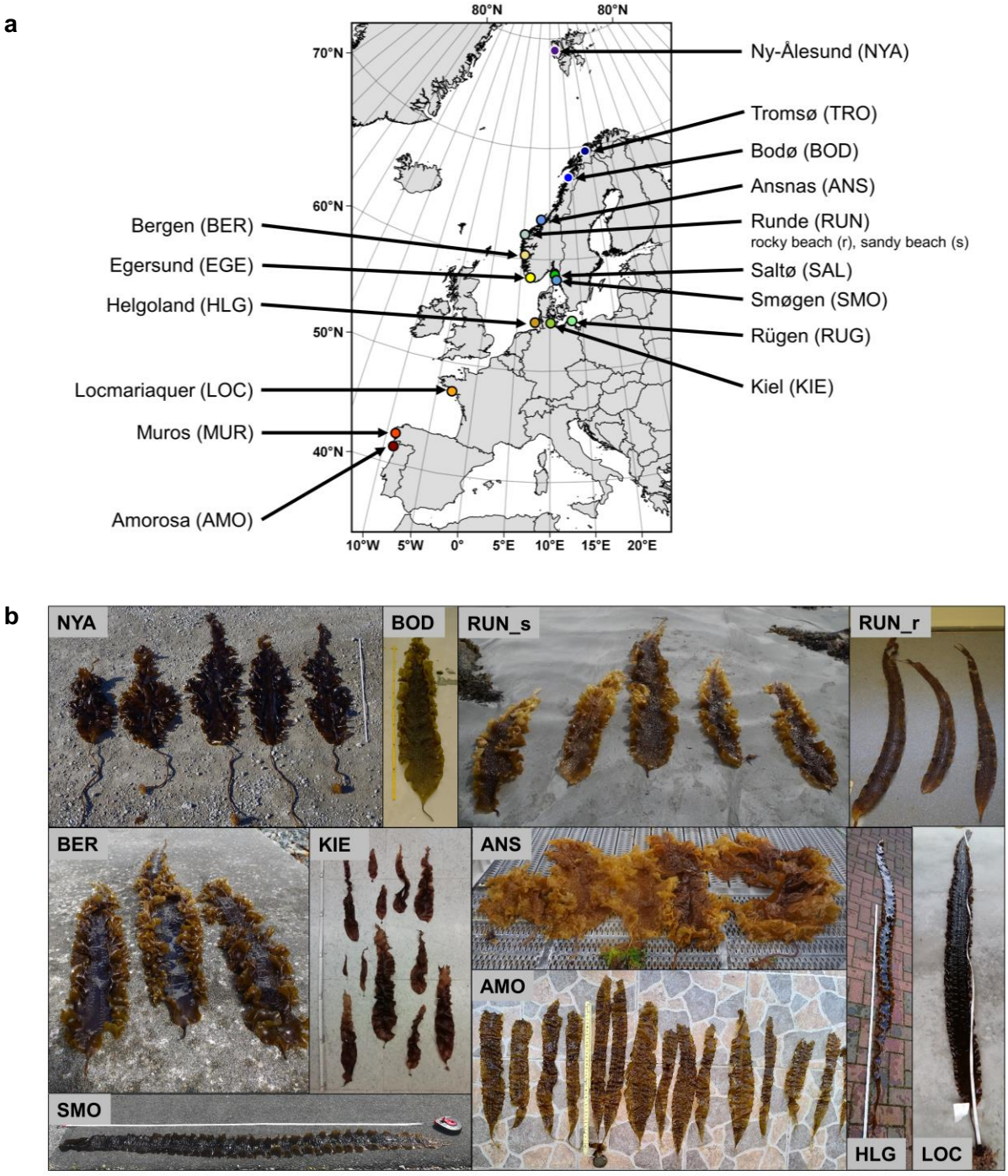


Fig. 5.1: Sampling of *Saccharina latissima* along the European coastline. **a:** Locations (map data: ©EuroGeographics for the administrative boundaries). **b:** Morphological variability of collected sporophytes.

Genetic analyses

DNA extraction, amplification and sequencing

DNA was extracted from dried tissue pieces of around 10 – 15 mm^2 with the NucleoSpin® Plant II Kit (Macherey-Nagel, Germany), applying minor adaptations to the manufacturers' protocol: The algal material was ground using dry silica sand and extra lysis buffer to dilute overly viscous samples. The cell lysis step was extended to a minimum of 1 h to allow for maximum DNA recovery. Lastly, an additional centrifugation step at 11,000 g (3 min) was applied before elution of the DNA. Raw DNA extracts were stored at −20 °C.

For sequence analyzes of the samples, the mitochondrial cytochrome-*c*-oxidase I gene (COI-5P) was amplified with the primer pair GazF2 and GazR2 (Lane et al. 2007). Primers were supplied by biomers.net (Germany).

Polymerase chain reactions (PCRs) contained 25 µL (12.5 µL MyTaq™ Mix [Bioline, Meridian Bioscience, Inc., USA], 8 µL of sterile H_2O, 1 µL of each primer [10 µM] and 2.5 µL DNA extract). DNA extracts were diluted with sterile H_2O at least to 1:10 or 1:100, to avoid inhibition of PCRs by high concentrations of impurities in the extract. The PCR program (Biometra® T Gradient thermocycler, Analytik Jana, Germany) was: 2 min at 94 °C, followed by 35 cycles of denaturation at 94 °C (30 s), annealing at 50 °C (1 min) and elongation at 72 °C (2 min), and a final extension step at 72 °C for 10 min. For subsequent sequencing, PCR products were purified either with the PureLink™ PCR Purification Kit (Invitrogen, ThermoFischer Scientific, Germany) or the SureClean Plus Kit (Bioline, Meridian Bioscience, Inc., USA), following the manufacturers' protocols. Purified products were sequenced commercially (Eurofins Genomics, Germany).

Phylogenetic analyses

Sequences were checked by eye in 4Peaks (Griekspoor & Tom Groothuis 2015) and aligned with PhyDE-1 v0.9971 (Müller et al. 2010). Initial species identification was confirmed by BLAST® searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and subsequent phylogenetic analysis of the COI-5P marker. The alignment comprised 618 bp and included published sequences of *S. latissima* as well as the close relative *S. japonica* (Areschoug) Lane et al., with *Hedophyllum* sequences serving as outgroup. A phylogenetic tree (**Fig. S5.1**, including representative sequences and GenBank accession numbers) based on the Maximum Likelihood (ML) criterion and a minimum spanning network among COI-5P haplotypes (**Fig. 5.2**) were constructed following the methods given in Heesch et al. (2020).

Principal Component Analysis (PCA)

Data acquisition: abiotic data, morphology and biochemical parameters

Sea surface temperature (SST) and salinity were recorded on the sampling day at each location (**Table 5.1**). Missing SST and salinity data for HLG and RUG were provided by the COSYNA system (Helmholtz-Zentrum Geesthacht, Zentrum für Material- und Küstenforschung GmbH; Breitbach et al. 2016 and Baschek et al. 2017). Missing abiotic data for MUR and AMO were retrieved as weekly means from the Giovanni online data system, developed and maintained by the NASA GES DISC (Acker & Leptoukh 2007). The samples and abiotic data from TRO were provided by a local aquaculture company (S. Matsson, Akvaplan-Niva). Additionally, the sampling depth respective to mean high tide (in categories: 0 – 1 m; 1 – 3 m; 3 – 5 m; > 5 m) was noted since we could not assure similar sampling depths at the different locations.

Frond morphology (length, width) was documented and the length to width ratio of the fronds (length:width) was calculated for each individual. For all biochemical analyzes, dried and ground meristematic material was used. Mannitol, C:N ratio and phlorotannins were measured as biochemical parameters, following the detailed descriptions in Diehl & Bischof (2021). All data are summarized in the supplements **Table S5.1**.

Statistics

The full data-set was split to analyze the morphological and biochemical diversity of *Saccharina latissima*. To evaluate patterns across the entire latitudinal range, only fully marine sampling locations were subset (latitudinal data-set, **Table 5.1**). The impacts along a salinity gradient were analyzed with samples collected in the Baltic Sea (Baltic data-set). The population from EGE was added as marine control from a similar latitude (**Table 5.1**).

Multivariate patterns were visualized with Principle Component Analyses (PCAs). PCAs were run using the prcomp-function in RStudio (version 4.0.0, Boston, MA, USA) and plotted as combined biplots and scatterplots with the ggbiplot function of the ggbiplot-package (Vu 2011). Missing values in the data-set were estimated based on cross-validation if needed, using the functions estim_ncpPCA and imputePCA of the missMDA-package (Josse & Husson 2016). All PCA biplot calculations followed this protocol. Pearson correlations were calculated using RStudio (version 4.0.0, Boston, MA, USA) and interpreted after Cohen (1988) to further analyze the links between biochemical and morphological parameters and local abiotic conditions (**Table S5.2**).

Table 5.1: Overview of the sampling details for *Saccharina latissima*. Subset data-sets: latitudinal (lat), Baltic (bal). Abiotic factors: sampling depth, sea surface temperature (SST) and salinity.

location			data-set	NE-coordinates		date	depth (m)	SST (°C)	salinity (S_A)
Norway, Spitsbergen	**Ny-Ålesund**	NYA	lat	78°55.496'	011°55.108'	28.06.2019	> 5	5.7	33
Norway	**Tromsø**	TRO	lat	69°45.259'	019°02.176'	28.06.2018	0 – 1	6.8	33[a]
Norway	**Bodø**	BOD	lat	67°16.591'	014°34.480'	17.06.2018	1 – 3	9.8	33
Norway	**Ansnas**	ANS	lat	63°38.271'	008°59.416'	20.06.2018	1 – 3	9.7	32
Norway	**Runde (sandy)**	RUN_s	lat	62°23.926'	005°39.288'	23.06.2018	0 – 1	13.1	32
Norway	**Runde (rocky)**	RUN_r	lat	62°24.199'	005°37.808'	23.06.2018	1 – 3	13.1	32
Norway	**Bergen**	BER	lat	60°13.615'	005°17.207'	26.06.2018	0 – 1	13.9	31
Norway	**Egersund**	EGE	lat, bal	58°27.035'	005°54.247'	10.07.2020	3 – 5	15	34
Germany, German Bight	**Helgoland**	HLG	lat	54°10.748'	007°55.068'	26.06.2018	3 – 5	15[b]	33[b]
France	**Locmariaquer**	LOC	lat	47°33.515'	002°55.468'	15.06.2018	3 – 5	20	32
Spain	**Muros**	MUR	lat	42°77.404'	-008°94.870'	07.07.2020	3 – 5	16.5[c]	35[c]
Portugal	**Amorosa**	AMO	lat	41°64.966'	-008°82.460'	02.07.2020	1 – 3	15[c]	35[c]
Sweden	**Saltø**	SAL	bal	58°52.455'	011°07'19.1''	21.06.2020	3 – 5	16	28
Sweden	**Smøgen**	SMO	bal	58°21.057'	011°13'37.2''	26.06.2020	3 – 5	20.6	21
Germany, Baltic Sea	**Kiel**	KIE	bal	54°25.563'	010°10.133'	06.06.2019	0 – 1	18.5	17
Germany, Baltic Sea	**Rügen**	RUG	bal	54°38.145'	013°25.381'	29.08.2019	> 5	18[b]	7[b]

[a] Akvaplan-Niva, Norway. [b] COSYNA system (Breitbach et al. 2016, Baschek et al. 2017). [c] Giovanni Satellite (Acker & Leptoukh 2007).

Results

Morphology and genetic diversity of Saccharina latissima *in Europe*

Morphological variability

The collected sporophytes varied considerably in their morphology (**Fig. 5.1b**). Many samples were ragged at the tip of the blade or started to decompose, hence, the actual length of the sporophyte was difficult to determine (**Table S5.1**). Regardless, strong variations in the morphological appearance of *Saccharina latissima* were observed between the different sampling locations. For instance, specimens from KIE and RUG in the Baltic Sea and those collected along the Iberian Coast (MUR, AMO) were considerably smaller than the other individuals. The length to width ratio (length:width) of samples varied between 1.4 (RUG) and 23.4. (HLG). Sporophytes from RUN_r (17.1 ± 4.0) and HLG (16.5 ± 2.3) were conspicuously long, narrow and thick. In contrast, the individuals collected in ANS (2.5 ± 0.4) were very wide, fragile and rigid. Even samples from very close locations showed marked differences: *S. latissima* specimens from the rocky shore in Runde (RUN_r) were – as mentioned above – very long and narrow, while samples from the same island, but collected at another shore (RUN_s), grew shorter and much wider (5.3 ± 1.7). Still, when plotting morphology alone (**Fig. S5.2**), no distinct patterns regarding distribution were observable, and the populations clustered densely together.

Genetic variability

Despite their morphological variability, all sporophytes were genetically identified as *Saccharina latissima*, apart from four specimens (TRO3, TRO5, BER1 and HLG1; **Table S5.1**), which consistently failed to amplify in PCRs. Samples that were included in the phylogenetic analysis formed a well-supported clade with other *S. latissima* (**Fig. S5.1**).

In the haplotype network (**Fig. 5.2**), no clear geographic clustering could be observed. Most samples belonged to one major haplotype closest to the European mitotype (in bright pink; reference) with one base pair difference. Some individuals from various locations differed from this main and the European mitotype by single substitutions, e.g. three samples from KIE and two from RUN, while all samples from HEL grouped with most of the southern samples, i.e. LOC, MUR and AMO, forming a separate clade. The Atlantic Arctic and Pacific Arctic mitotypes (ref) differed from the main haplotype by four and five substitutions, respectively.

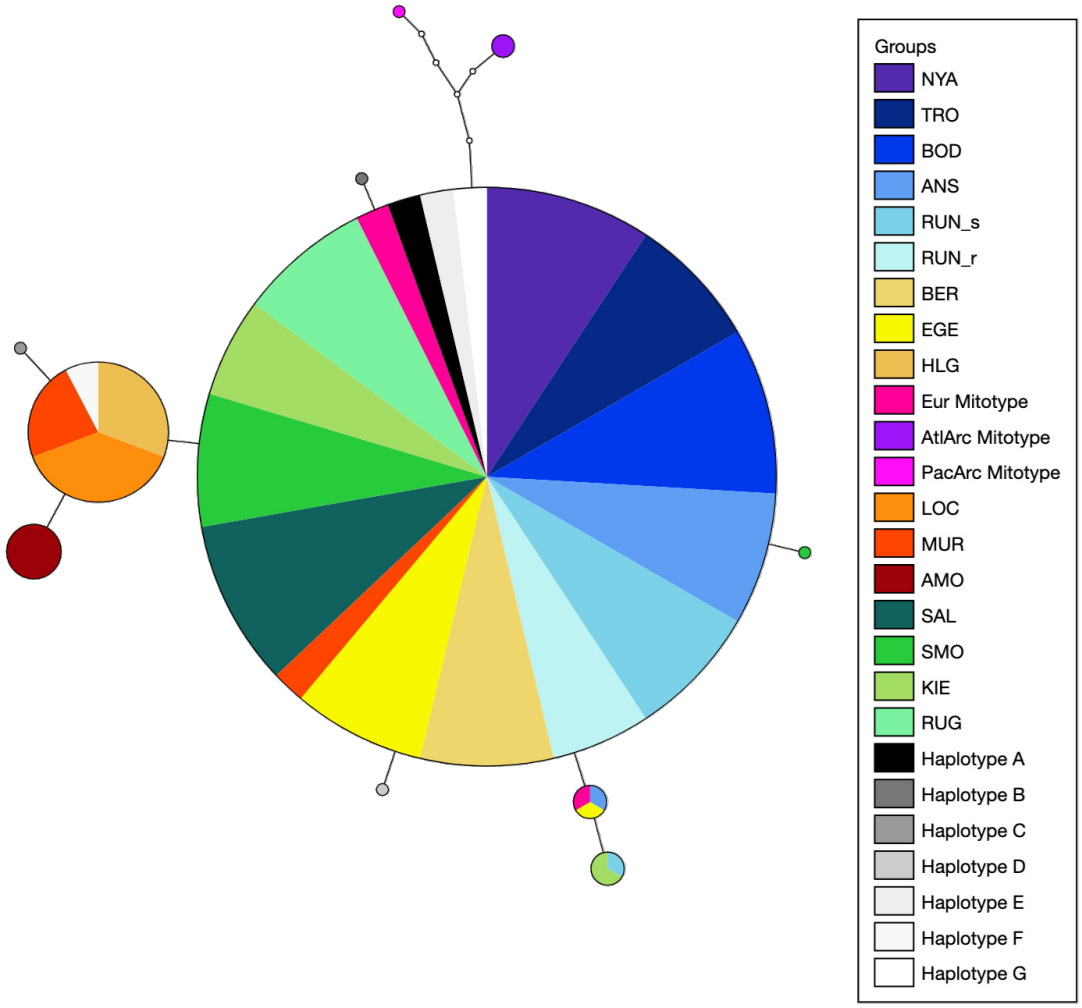


Fig. 5.2: Haplotype network based on COI-5P sequences of *Saccharina latissima*. Open dots refer to base changes between encountered haplotypes (full circles); sizes of circles are proportional to numbers of sequences included. Haplotypes in gray scales are based on Luttikhuizen et al. (2018); light pink to light purple scales represent mitotypes based on McDevit & Saunders (2009, 2010) (see **Fig. S5.1** for references).

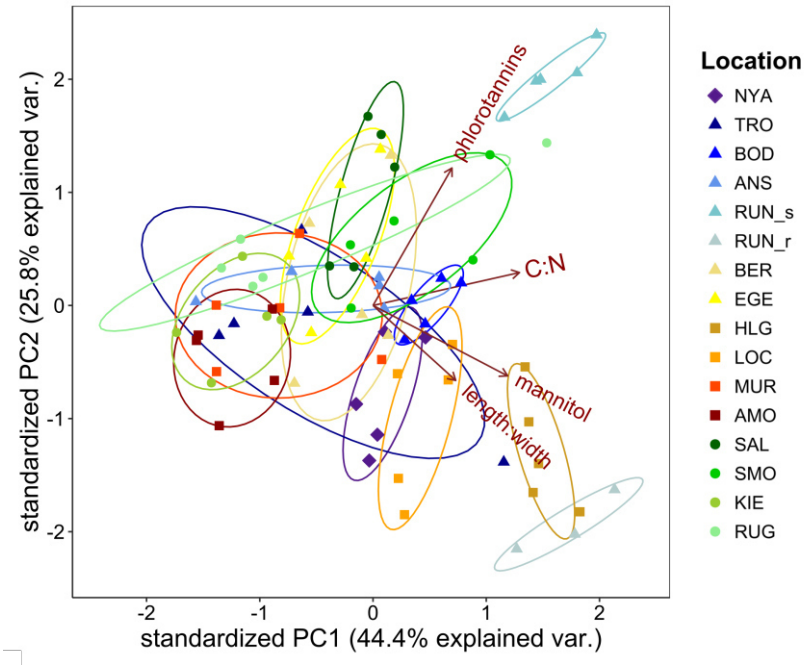


Fig. 5.3: Combined score- & biplot of the first two principal components (PC) of the principal component analyses (PCA) performed on samples of *Saccharina latissima* from different sample locations along the European coast. Plotted are the length to width ratio (length:width) and biochemical compounds (mannitol, carbon to nitrogen ratio [C:N], phlorotannins). The arrows represent the loadings of each PCA. Sampling locations are indicated by different colors. Different shapes represent hydrographic regions of the samples: ♦ = Arctic (Spitsbergen), ▲ = Norwegian Sea, ■ = North Sea / North Atlantic, ● = Baltic Sea. Each point represents one individual.

Saccharina latissima *across latitudes*

The results of all calculated correlations are listed in **Table S5.2** in the supplement.

Morphology vs. abiotic data. Regarding SST, a negative correlation was determined for sporophyte width ($r = -0.51$, $p < 0.001$) and positive correlation for the ratio of length:width ($r = 0.40$, $p < 0.01$), meaning sporophytes grew overall wider in colder regions (**Table S5.2**). There were weak negative correlations observable between salinity and both length ($r = -0.31$, $p < 0.05$) and width ($r = -0.37$, $p < 0.001$), thus length:width was not affected. Samples were generally smaller at higher salinity. Since all populations grew under fully marine conditions (S_A 31 – 35), the significant correlation can be explained by the considerably smaller sporophytes sampled in MUR and AMO at the highest salinity (**Table 5.1**). Across latitudes, the different depths at the sampling locations had no significant impact on the morphological appearance in *S. latissima*.

Biochemistry vs. abiotic data. To investigate the impact of the abiotic conditions on the biochemical profile of *S. latissima*, each parameter was correlated separately (**Table S5.2**). For mannitol, no significant correlations were detected with SST or sampling depth, but it correlated negatively with salinity ($r = -0.36$, $p < 0.01$), exhibiting lower mannitol concentrations at higher salinities. Higher SST led to higher total C as well as higher total nitrogen (N) concentrations ($r_C = 0.30$, $p_C < 0.5$; $r_N = 0.35$, $p_N < 0.01$), resulting in an unchanged C:N ratio. However, salinity showed a significant negative correlation ($r = -0.55$, $p < 0.001$) with C:N. Lower C:N were measured in the populations with higher salinity, although this can be entirely ascribed to the high N concentrations in the samples from MUR and AMO (S_A 35) ($r = -0.69$, $p < 0.001$). Comparable to mannitol, sampling depth did not correlate with C:N, total C or total N. Phlorotannin contents were significantly correlated to sampling depth ($r = -0.33$, $p < 0.05$), with lower concentrations measured in samples from greater depth, but there was no correlation with salinity or SST.

Morphology vs. biochemistry. Positive correlations were detected between mannitol and length ($r = 0.44$, $p < 0.001$) though not width, which resulted in positive correlation of mannitol and length:width ($r = 0.37$, $p < 0.01$) (**Table S5.2**). C:N showed a weak positive correlation with length ($r = 0.29$, $p < 0.05$), but since no correlation with width was observed, the impact of length was too small to be reflected in the length:width ratio. Total C did not correlate with size, thus, variation in C:N was entirely based on the reduced total N in larger sporophytes

(r_{length} = −0.44, p_{length} < 0.001; r_{width} = −0.47, p_{width} < 0.001). For phlorotannins, no correlations with morphological parameters were determined across latitudes.

Saccharina latissima *along a salinity gradient*

The results of all calculated correlations are listed in **Table S5.2** in the supplement.

Morphology vs. abiotic data. Strong positive correlations were found between salinity and length (r = 0.61, p < 0.01), and width (r = 0.88, p < 0.001) (**Table S5.2**). Samples were considerably smaller at lower salinities, however, length:width did not change with salinity. Comparable to the latitudinal data, SST correlated negatively with the width of the sporophytes (r_{width} = −0.51, p_{width} < 0.01; $r_{length:width}$ = 0.48, $p_{length:width}$ < 0.05), with samples growing wider at colder locations. The morphological appearance was not affected by different sampling depth along the salinity gradient.

Biochemistry vs. abiotic data. Mannitol strongly correlated with salinity (r = 0.66, p < 0.001) but not with SST or depth (**Table S5.2**). Contrary to the latitudinal data-set, the concentration was significantly lower in the populations with low salinity. There was no correlation between salinity or SST, however, the C:N revealed a positive correlation with the sampling depths at the different locations (r = 0.50, p < 0.05), since total C correlated positively (r = 0.64, p < 0.001) and total N correlated negatively with depth (r = −0.41, p < 0.05). Phlorotannins were not significantly correlated to any of the three abiotic parameters.

Morphology vs. biochemistry. Positive correlations were detected between mannitol and both length (r = 0.67, p < 0.001) and width (r = 0.56, p < 0.01), leading to balanced length:width ratios, even though the overall size significantly decreased at lower salinities. C:N also correlated positively with both length (r = 0.45, p < 0.05) and width (r = 0.44, p < 0.05), and accordingly, no correlation with length:width was found. Comparable to the latitudinal data-set, total N decreased with increasing size (r_{length} = −0.48, p_{length} < 0.05; r_{width} = −0.47, p_{width} < 0.05). Furthermore, a positive correlation was found with total C (r = 0.42, p < 0.05). However, these differences were not reflected in a correlation between C:N, total C or total N and length:width. Regarding phlorotannins, a correlation with length (r = 0.57, p < 0.01) was determined, which was also reflected in the correlation between phlorotannins and length:width (r = 0.54, p < 0.01).

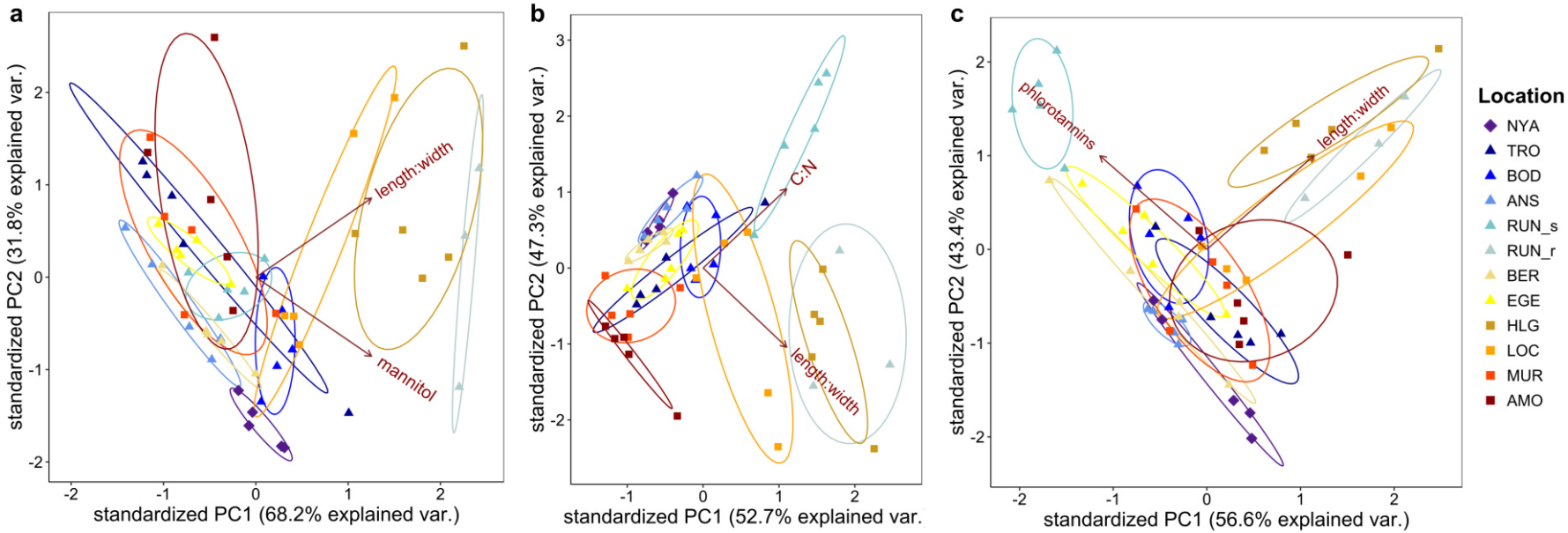
Habitat-specificity of biochemical profiles

To explore potential habitat-specific biochemical profiles of *S. latissima*, we first plotted the three biochemical parameters together (**Fig. S5.3**). Dispersion of the individual sporophytes within the populations was very high, and no pattern of the populations regarding their distribution was distinguishable. However, the above results clearly show that morphology was also dependent on different abiotic conditions and had a significant effect on the biochemical profile of *S. latissima*. Individual effects of length and width were overall well reflected by the impact of length:width on mannitol, C:N and phlorotannins. Thus, to exclude significant implications of morphology on the biochemical profile, we combined the morphology (length:width) and the biochemical composition of *S. latissima* in the PCA plot (**Fig. 5.3**). Most populations clustered closely together. Only HLG and RUN_r, which were both conspicuously long, and RUN_s (**Fig. 5.1b**), collected at a sandy beach, were slightly separated from the other populations. Also, after separating the two data-sets, no patterns reflecting the distribution across latitudes or along a salinity gradient were detectable. Instead, the dispersion between and within the populations was very high (data not shown). Consequentially, no distinction in the overall biochemical profiles of the populations in terms of their distribution could be determined.

To reveal whether mannitol, C:N or phlorotannins alone support site-specific differentiations, these parameters were also plotted individually and separately for each data-set. In neither of the three parameters, clear distinct patterns regarding distribution were displayed in the latitudinal data-set (**Fig. 5.4**). However, based on the length:width ratio, LOC, and even more so HLG and RUN_r separated from the densely clustered other populations in all three plots. Furthermore, in **Figure 5.4b** and **5.4c**, RUN_s also separated from the other populations, based on contents of C:N and phlorotannins. Phlorotannins and mannitol are high-carbon molecules, thus, to reveal potential impact on C:N, we correlated their concentrations with the total carbon content (C). We found a positive correlation between phlorotannins and total C ($r = 0.46$, $p < 0.001$), but no correlation between mannitol and total C (**Table S5.2**), explaining the missing separation of RUN_s in **Figure 5.4a**.

No clear patterns were distinguishable for mannitol, C:N or phlorotannins in the Baltic samples, and dispersion between and within the population was high (**Fig. S5.4**). Although size of the specimens considerably decreased along the Baltic Sea, the fully marine control population from EGE did not separate from the others as length:width of the individuals was similar. Thus, morphological differences in size were not reflected in the biochemical signatures.

Fig. 5.4: Combined score- & biplot of the principal component analyses (PCA) performed on samples of *Saccharina latissima* from different sample locations along a salinity gradient. Plotted are the length to width ratio (length:width) and the biochemical compounds individually. **a:** Mannitol. **b:** Carbon to nitrogen ratio (C:N). **c:** Phlorotannins. The arrows represent the loadings of each PCA. Sampling locations are indicated by different colors. Different shapes represent hydrographic regions of the samples: ♦ = Arctic (Spitsbergen), ▲ = Norwegian Sea, ■ = North Sea / North Atlantic. Each point represents one individual.



Discussion

This study is the first approach to address the lack of comprehensive data-sets on kelps which integrate morphological, biochemical, genetic and geographic data (Bolton 2010). We provide important information on the intraspecific variability of the broadly distributed *Saccharina latissima* within Europe. Considerable imprints of local abiotic conditions on the morphology and the biochemical composition of *S. latissima* were detected. However, contrary to our hypotheses, no clear geographical patterns or links between biochemical profiles and haplotypes could be detected.

Morphological profiles along gradients

In our study, the morphology of *Saccharina latissima* was highly influenced by various abiotic conditions. We found clear evidence that salinity strongly affected the size of the field sporophytes (Lüning 1990). In accordance with the optimum salinity growth range between S_A 23 and 31 (Gerard et al. 1987), samples across the Baltic Sea greatly decreased in size along the salinity gradient. Additionally, high salinity at MUR and AMO (S_A 35) probably diminished growth in the field sporophytes. However, in MUR also larger individuals were found in larger depths of the region (C. Monteiro pers. comm.).

Apart from salinity, the effect of temperature on the size should not be underestimated. Correlations between morphological appearance and sea surface temperature (SST) were found both along latitudes and along the salinity gradient. Sporophytes grew wider, thus, had a smaller length to width ratio (length:width) in colder regions. A seasonal increase in width has previously been observed during summer and fall in *S. latissima* from Norway (Sjøtun 1993). Accordingly, the seasonal impact on growth activity across latitudes (Diehl et al. submitted), mirrored by different SST in this study, potentially resulted in changes in the ratio between frond length and width. Different length:width ratios have also been reported from individuals of different ages (Sjøtun 1993). Hence, age might also have influenced the morphological appearance of our samples, even though we aimed to collect the largest specimens, and specimens of equal size at each location. Additionally, wave exposure has long been known to strongly influence the morphology of kelps, affecting blade, stipe and holdfast (Gerard 1987, Lüning 1990, Fowler-Walker et al. 2006, Coppin et al. 2020). In more exposed habitats sporophytes of *S. latissima* form narrow blades with solid stipes, while they exhibit broad blades with hollow stipes in more sheltered habitats (Lüning 1990). Our observations

are in accordance with those studies, e.g. the sampling location in ANS was situated in a very calm bay, while HLG and RUN_r were both rather wind- and wave-exposed habitats. Regardless of their latitudinal distribution, the morphology of our specimens appeared to be mostly affected by exposure, although we cannot clearly clarify this.

Biochemical profiles along gradients

The impacts of different abiotic factors on the morphology and biochemistry of kelps are well studied (Lüning 1990, Amsler 2008, Wiencke & Bischof 2012 and references therein). However, to our knowledge, this study is the first to investigate links between morphology and biochemical composition on a large geographical scale. Different stress responses of cultivated and field-grown sporophytes of *S. latissima* may be caused by age-dependent and morpho-functional features (Heinrich et al. 2016). Consequently, the impact of abiotic drivers in the field may also differ depending on the morphology of a sporophyte.

As mannitol is the main photosynthetic product in Phaeophyceae, its concentration is directly affected by solar radiation (Gylle et al. 2009), which varies across latitudes and within the water column. Mannitol may moreover be influenced by variability in temperature (Iwamoto & Shiraiwa 2005, Ji et al. 2016, Diehl et al. 2020), as it has, for example, been proposed as an anti-freezing compound in *S. latissima* (Monteiro et al. 2021). However, we did not detect significant correlations between mannitol and SST or sampling depth, neither across latitudes nor in the Baltic data-set. Instead, mannitol concentrations in *S. latissima* were strongly affected by ambient salinity. Mannitol acts as an osmolyte and 'compatible solute' under osmotic stress (Kirst 1989, Karsten 2012). In kelps, its concentrations vary under different salinities: lower salinity results in lower intracellular osmotic stress, and as a consequence, less mannitol is stored (Diehl et al. 2020, Monteiro et al. 2021). We could confirm this effect on mannitol content in field-grown sporophytes along the strong salinity gradient across the Baltic Sea. However, in the latitudinal data-set, a contrasting correlation was detected, with higher salinities across latitudes seemingly resulting in lower mannitol concentrations. Mannitol per gram dry weight increased with the overall size of the sporophytes in this study. The storage of carbohydrates, such as mannitol, is dependent on growth activity. I.e., during slow growth periods, fully-grown sporophytes may accumulate mannitol (Johnston et al. 1977, Schiener et al. 2015). Thus, decreasing size and increasing depth along the salinity gradient in the Baltic Sea might have additionally affected mannitol concentrations. Furthermore, also

samples from MUR and AMO were considerably smaller. Accordingly, low mannitol content in these samples could be ascribed to their size and not to the high salinity of their habitat.

The ratio between total carbon (C) and total nitrogen (N) content (C:N) is usually used to monitor the nutrient status of seaweeds (Hurd et al. 2014). We found no impact of length:width on C:N, total C or total N. However, an impact of temperature on nutrient uptake has been reported for brown algae (Graiff et al. 2015, Roleda & Hurd 2019), but also an impact of salinity (Jiménez & Niell 1991, Gordillo et al. 2002), and interaction between temperature and salinity on N uptake has been proposed for seaweeds (Mandal et al. 2015, Diehl et al. 2020). In this study, C:N ratios of *S. latissima* were not affected by ambient SST since both total C and total N increased at higher temperatures in the latitudinal data-set and samples, while samples in the Baltic data-set were not affected by SST. Contrary to Nielsen et al. (2016a), we did not detect decreasing total C with increasing salinities across the salinity gradient in the Baltic Sea and also did not find increasing total N at hyposalinity (Diehl et al. 2020). Instead, we observed a correlation of total C and total N with sampling depth in the Baltic Sea, but not along latitudes, which most probably can be attributed to the size of the samples. Moreover, we found a putative correlation with salinity in the latitudinal data-set. In the smallest samples from MUR and AMO, where the highest salinities were measured, C:N was particularly low due to high total N. However, the actual impact of salinity seems questionable since no effect of salinity was determined along the strong salinity gradient in the Baltic Sea. On the other hand, the Iberian Coast is characterized by upwelling events, during which cold and nutrient-rich deep waters reach the sea surface (Lourenço et al. 2016). Accordingly, C:N in the populations of MUR and AMO was strongly affected by high ambient nutrient concentrations rather than by salinity or morphology. Summarizing, potential effects of morphology, salinity and SST in nature are excelled by nutrient conditions. While *S. latissima* showed acclimation to different temperature and salinity conditions within 24 h (Li et al. 2020, Monteiro et al. 2021), nutrients can be stored in high concentrations for times when they are limited. Hence, the nutrient concentration in the environment is a critical factor for the morphological appearance and biochemical composition of *S. latissima* (Lüning 1990). However, due to the geographical spread of our study sites, nutrient monitoring was unfortunately beyond the scope of our study.

Nevertheless, C:N is also affected by other factors, as total C constitutes all carbohydrates, including alginate, cellulose and other polysaccharides, and is influenced by the tissue structure (Peters et al. 2005, Amsler 2008). While we observed varying amounts of mucilage in the sporophytes during sampling, suggesting differing polysaccharide contents and consequently

total C levels, different C:N ratios in our sampling campaign were mainly based on lower total N content and not on increased total C with increasing size.

Still, we found a positive correlation between total C, and thus C:N, and phlorotannins. Phlorotannins are important components of cell walls (Schoenwaelder & Clayton 1999), enhancing tissue strength (Simonson et al. 2015). In our study, this was confirmed for samples along the salinity gradient, where larger and sturdier individuals contained higher phlorotannin concentrations. However, for large sporophytes found along the latitudinal gradient, the morphology did not appear to be impacted by phlorotannins, even though the haptic of several sporophytes differed greatly, e.g. HLG and ANS. Phlorotannins do also serve as photo-protective compounds in brown algae (Amsler 2008). However, sampling depth did not significantly correlate with phlorotannins in the Baltic populations. Nevertheless, in the latitudinal dataset, a weak negative correlation was found regarding depth. The light regime varies across latitudes and water depths, but it is also affected, for instance, by tidal range, sedimentation or day length (Morel 1991, Hanelt et al. 2001, Rozema et al. 2002). Sporophytes of *S. latissima* growing in dense kelp forests such as at RUN_r, HLG or LOC were shaded and therefore contained comparatively low amounts of phlorotannins (**Table S5.1**). On the other hand, extraordinarily high phlorotannin concentrations were measured in samples from RUN_s, which also resulted in a separation in the PCA plot (**Fig. 5.4**). These results may be explained by their habitat since these samples grew on solitary rocks surrounded by a sandy habitat. With its bright surface, sand reflects incoming radiation (Chadyšiene & Girgždys 2008). This albedo effect may result in higher light stress for the surrounding sporophytes, and consequently, phlorotannin concentration may be higher compared to other populations.

Genetic diversity

Bolton (2010) highlighted the importance of combining morphological, ecological and genetic analyses for our understanding of the evolution of kelps, especially in widespread taxa, such as the genus *Saccharina*. Camus et al. (2018), for instance, investigated the genetic and phenotypic diversity of *Macrocystis pyrifera* and determined links between genetic clusters along latitudinal distribution, morphological diversity and variations in environmental drivers. By assessing the intraspecific variability on several levels, the process of ecotypic separation across Europe, as it is currently proposed, might be better understood.

While genetic sequence comparisons consistently confirmed identifications of our specimens as *S. latissima*, the haplotype network failed to separate distinct genetic clusters according to

geographic distribution. While populations from the Baltic Sea did not separate from fully marine populations, the southern populations from HLG to AMO had a slightly different haplotype, in accordance with prior studies (Luttikhuizen et al. 2018, Neiva et al. 2018). In the North East Atlantic, *S. latissima* can be divided into two phylogenetic groups: the northern cluster ranges from Spitsbergen to southern Norway and the southern one includes Brittany and the Iberian Coast (Neiva et al. 2018). Luttikhuizen et al. (2018) reported close relationships between the different populations but also slight differentiation between samples from southern Norway and Brittany within a haplotype network based on the COI marker. However, in general, microsatellite markers are more sensitive. For example, Nielsen et al. (2016b) used microsatellites to separate *S. latissima* populations from the Baltic Sea, and Guzinski et al. (2020) investigated genetic connectivity of *S. latissima* populations across the same latitudinal distribution range as in the present study. While they did not observe significant variations in genetic diversity with latitude, they demonstrated a high degree of inter-population differentiation, indicating that this kind of marker is more suited to complement intraspecific studies on biochemical and morphological variability.

Habitat-specific signatures in Saccharina latissima*?*

Different populations are often treated as homogenous physiological units, independent of the species' distribution (Reed et al. 2011). However, a direct comparison of different populations of the same kelp species is difficult as they exhibit habitat-specific physiological traits (King et al. 2018, Liesner et al. 2020, Martins et al. 2020, Diehl et al. submitted). For that reason, we aimed to characterize habitat-specific biochemical signatures *S. latissima*.

Mannitol, C:N and phlorotannins are among the most important compounds reflecting stress responses in *S. latissima* and are strongly affected by the monitored abiotic parameters (Lüning 1990, Nielsen et al. 2016a, Manns et al. 2017, Coppin et al. 2020). Confirming the results of these previous studies, we detected strong dependencies of the analyzed traits on the local abiotic conditions, i.e. the individual profiles of specific biochemical parameters in *S. latissima* corresponded to respective environmental conditions (Martins et al. 2014, Monteiro et al. 2020). We also determined a significant impact of morphology on the biochemical profiles and therefore included both morphological and biochemical phenotypes to detect habitat-specific biochemical signatures in *S. latissima*. However, contrary to our hypothesis, the biochemical profiles in our study did not pinpoint the geographical origin on larger scales,

as *S. latissima* did not exhibit habitat-specific biochemical signatures. Still, some trends towards habitat-specific partition of *S. latissima* populations occur independently from their geographical distribution. For instance, based on morphological traits, the populations from HLG and RUN_r were clearly separated, even more than the population from LOC. However, to actually reveal habitat-specific biochemical signatures, corresponding to the geographical distribution, more specific biochemical characteristics may be needed, e.g. unique lipidomic profiles such as observed in *S. latissima* from France, Great Britain and Norway (Monteiro et al. 2020).

While major impacts of salinity were determined on morphology and biochemical profile of *S. latissima*, the Baltic samples did not clearly separate from the fully marine populations. Due to the high density of saltwater from the North Sea which enters the Baltic Sea in the Skagerrak, salinities in the Baltic Sea are generally higher at greater depths, where *S. latissima* descends to (Nielsen et al. 2016a). On the other hand, greater water depths equal lower irradiance, and hence limited growth and variation in biochemical composition (Gerard 1988, Spurkland & Iken 2011, Endo et al. 2017). Thus, besides a direct impact on the osmotic status of the species, salinity, especially hyposalinity, seems to also have indirect influences on biochemical composition and complicates the indication of habitat-specific traits.

Long-term selective effects can generate genetic diversity along latitudinal gradients, eventually resulting in the emergence of ecotypes (Parmesan 2006, Nicotra et al. 2010, King et al. 2018). These comprise populations that are locally adapted and thus phenotypically and genetically differentiated from specimens of other geographic regions (Conner & Hartl 2004). Gene flow between populations generally depends on the dispersal capacity of a species, which is known to be limited in kelps compared to other marine organisms, for instance due to the absence of rocky substrates in potential new habitats and other dispersal barriers (Bolton 2010, Valero et al. 2011). Luttikhuizen et al. (2018) suggest that intraspecific genetic differentiation of *S. latissima* in Europe is expected to increase, if population structures are left undisturbed, since the equilibrium of population differentiation are not yet reached. Hence, a further distinction of populations and the accomplishment of equilibrium would support the evolution of ecotypes or even further genetic differentiation within the species complex (Luttikhuizen et al. 2018).

We did not detect distinct haplotypes in the sampled *S. latissima* populations, thus supporting the afore-mentioned hypothesis. Indeed, the fact that we could not identify habitat-specific traits and that the evaluated parameters were highly plastic and correlated with the abiotic conditions, rather than with geographical origin, suggests traits of high phenotypic plasticity

in *S. latissima* instead of the existence of genetically fixed ecotypes. Nevertheless, traits of high plasticity might also be exhibited by ecotypically differentiated species, even though they are genetically fixed to a local optimum (de Jong 2005). In fact, the separation of the southernmost populations in our haplotype network supports the proposal that intraspecific separation of *S. latissima* is still an ongoing process and not completed yet (Luttikhuizen et al. 2018). Genetic and ecotypic differentiation could be intermitted by climatic changes, e.g. by rising temperatures and hyposalinity, which impose increasing threats on marine ecosystems (IPCC 2019). The expected shift in macroalgal communities, as a result of changing habitats (Müller et al. 2009, Assis et al. 2018), will potentially disturb population structures and hence, speciation.

Summarizing, even though no habitat-specific biochemical profiles of combined mannitol, C:N ratio and phlorotannins could be established in our study, marked imprints of abiotic conditions on the biochemical composition of *S. latissima* were detected. In the Baltic Sea, no genetic or phenotypic clusters were observed. Differentiations have not manifested genetically and were not reflected in COI-5P diversity. While we are aware that our conclusions are based on small sampling sizes at each location, we can conclude that this study provides a basis for subsequent studies assessing the intraspecific diversity of *S. latissima*. For more comprehensive results, molecular analyses based on microsatellite markers are essential in future studies on combined genetic and phenotypic diversity.

Acknowledgments

The authors are very grateful to the colleagues helping to collect *Saccharina latissima* all over Europe: S. Matsson (Akvaplan-Niva, Norway; with support from the MACROSEA project: RCN project254883) in Tromsø, M. Roleda in Bodø, S. Steinhagen in Egersund, Saltø und Smøgen, K. Romoth in Rügen, A. Wagner in Helgoland, M. Valero, C. Destombe and L. Fouqueau in Locmariaquer and C. Monteiro in Muro and Amorosa. Thank you for the support at the field stations in Runde, Bergen and Ny-Ålesund. Abiotic data from the Iberian Coast used in this paper were produced with the Giovanni online data system, developed and maintained by the NASA GES DISC. Sampling in France was conducted in accordance with the French legislation on the Access to Genetic Resources and Benefit-Sharing. Thank you to the Scientific Diving Teams in Ny-Ålesund and on Helgoland for the help in collecting. B. Meyer-Schlosser (University of Bremen) supported the sampling campaign in Norway 2018 and the pigment analyses. J. Müller (University of Rostock) ran the C:N analyses. Thank you to Kellner & Stoll – Stiftung für Klima und Umwelt for funding the genetic analyses. This study was further supported by the 2015 – 2016 BiodivERsA COFUND call for research proposals (program MARFOR), with the national funder German Research Foundation (DFG; grant no. VA 105/25-1). S.H. acknowledges support from the DFG via the SPP 1158 (Coordination grant to U. Karsten, University of Rostock).

References

Acker JG, Leptoukh G (2007) Giovanni Satellite: Online analysis enhances use of NASA Earth Science Data. Eos, *Trans Am Geophys Union* 88:14–17.

Amsler CD (2008) Algal chemical ecology. Amsler CD (ed) Springer-Verlag Berlin Heidelberg.

Araújo RM, Assis J, Aguillar R, Airoldi L, Bárbara I, Bartsch I, Bekkby T, Christie H, Davoult D, Derrien-Courtel S, Fernandez C, Fredriksen S, Gevaert F, Gundersen H, Le Gal A, Lévêque L, Mieszkowska N, Norderhaug KM, Oliveira P, Puente A, Rico JM, Rinde E, Schubert H, Strain EM, Valero M, Viard F, Sousa-Pinto I (2016) Status, trends and drivers of kelp forests in Europe: An expert assessment. *Biodivers Conserv* 25:1319–1348.

Assis J, Araújo MB, Serrão EA (2018) Projected climate changes threaten ancient refugia of kelp forests in the North Atlantic. *Glob Chang Biol* 24:e55–e66.

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J (2008) The genus *Laminaria sensu lato*: Recent insights and developments. *Eur J Phycol* 43:1–86.

Baschek B, Schroeder F, Brix H, Riethmüller R, Badewien TH, Breitbach G, Brügge B, Colijn F, Doerffer R, Eschenbach C, Friedrich J, Fischer P, Garthe S, Horstmann J, Krasemann H, Metfies K, Merckelbach L, Ohle N, Petersen W, Pröfrock D, Röttgers R, Schlüter M, Schulz J, Schulz-Stellenfleth J, Stanev E, Staneva J, Winter C, Wirtz K, Wollschläger J, Zielinski O, Ziemer F (2017) The Coastal observing system for Northern and Arctic seas (COSYNA). *Ocean Sci* 13:379–410.

Bekkby T, Moy FE (2011) Developing spatial models of sugar kelp (*Saccharina latissima*) potential distribution under natural conditions and areas of its disappearance in Skagerrak. *Estuar Coast Shelf Sci* 95:477–483.

Bischof K, Buschbaum C, Fredriksen S, Gordillo FJL, Heinrich S, Jiménez C, Lütz C, Molis M, Roleda MY, Schwanitz M, Wiencke C (2019) Kelps and environmental changes in Kongsfjorden: Stress perception and responses. In: *The ecosystem of Kongsfjorden, Svalbard. Advances in polar ecology*. Hop H, Wiencke C (eds) Springer International, Published AG, Switzerland, Cham, p 373–422.

Bischof K, Hanelt D, Tüg H, Karsten U, Brouwer PEM, Wiencke C (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biol* 20:388–395.

Bolton JJ (2010) The biogeography of kelps (Laminariales, Phaeophyceae): A global analysis with new insights from recent advances in molecular phylogenetics. *Helgol Mar Res* 64:263–279.

Bolton JJ, Lüning K (1982) Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. Mar Biol 66:89–94.

Breitbach G, Krasemann H, Behr D, Beringer S, Lange U, Vo N, Schroeder F (2016) Accessing diverse data comprehensively - CODM, the COSYNA data portal. Ocean Sci 12:909–923.

Camus C, Faugeron S, Buschmann AH (2018) Assessment of genetic and phenotypic diversity of the giant kelp, *Macrocystis pyrifera*, to support breeding programs. *Algal Res* 30:101–112.

Chadyšiene R, Girgždys A (2008) Ultraviolet radiation albedo of natural surfaces. *J Environ Eng Landsc Manag* 16:83–88.

Cohen J (1988) Statistical power analysis for the behavioral sciences, Second Edi. Lawrence Erlbaum Associates, United States of America, New York.

Conner JK, Hartl D. (2004) A primer of ecological genetics. Sinauer Associates Incorporated, Sunderland, USA.

Coppin R, Rautenbach C, Ponton TJ, Smit AJ (2020) Investigating waves and temperature as drivers of kelp morphology. *Front Mar Sci* 7:567.

Dayton PK (1985) Ecology of kelp communities. *Annu Rev Ecol Syst* 16:215–245.

Diehl N, Bischof K (2021) Coping with a changing Arctic: mechanisms of acclimation in the brown seaweed *Saccharina latissima* from Spitsbergen. *Mar Ecol Prog Ser* 657:43–57.

Diehl N, Karsten U, Bischof K (2020) Impacts of combined temperature and salinity stress on the endemic Arctic brown seaweed *Laminaria solidungula* J. Agardh. *Polar Biol* 43:647–656.

Diehl N, Roleda MY, Bartsch I, Karsten U, Bischof K (submitted) Summer heatwave impacts on the European kelp *Saccharina latissima* across its latitudinal distribution gradient. *Front Mar Sci* submitted April 2021.

Endo H, Suehiro K, Gao X, Agatsuma Y (2017) Interactive effects of elevated summer temperature, nutrient availability, and irradiance on growth and chemical compositions of juvenile kelp, *Eisenia bicyclis*. *Phycol Res* 65:118–126.

Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgol Meeresunt* 34:15–29.

Fowler-Walker MJ, Wernberg T, Connell SD (2006) Differences in kelp morphology between wave sheltered and exposed localities: morphologically plastic or fixed traits? *Mar Biol* 148:755–767.

Gerard VA (1988) Ecotypic differentiation in light-related traits of the kelp *Laminaria saccharina*. *Mar Biol* 97:25–36.

Gerard VA (1987) Hydrodynamic streamlining of *Laminaria saccharina* Lamour. in response to mechanical stress. *J Exp Mar Bio Ecol* 107:237–244.

Gerard VA, DuBois K, Greene R (1987) Growth responses of two *Laminaria saccharina* populations to environmental variation. *Hydrobiologia* 151/152:229–232.

Gordillo FJL, Dring MJ, Savidge G (2002) Nitrate and phosphate uptake characteristics of three species of brown algae cultured at low salinity. *Mar Ecol Prog Ser* 234:111–118.

Graiff A, Bartsch I, Ruth W, Wahl M, Karsten U (2015) Season exerts differential effects of ocean acidification and warming on growth and carbon metabolism of the seaweed *Fucus vesiculosus* in the western Baltic Sea. *Front Mar Sci* 2:112.

Griekspoor A, Tom Groothuis T (2015) 4Peaks. https://www.nucleobytes.com/.

Guzinski J, Mauger S, Cock JM, Valero M (2016) Characterization of newly developed expressed sequence tag-derived microsatellite markers revealed low genetic diversity within and low connectivity between European *Saccharina latissima* populations. *J Appl Phycol* 28:3057–3070.

Guzinski J, Ruggeri P, Ballenghien M, Mauger S, Jacquemin B, Jollivet C, Coudret J, Jaugeon L, Destombe C, Valero M (2020) Seascape genomics of the sugar kelp *Saccharina latissima* along the north eastern Atlantic latitudinal gradient. *Genes* 11:1503.

Gylle AM, Nygard CA, Ekelund NGA (2009) Desiccation and salinity effects on marine and brackish *Fucus vesiculosus* L. (Phaeophyceae). *Phycologia* 48:156–164.

Hanelt D, Tüg H, Bischof K, Groß C, Lippert H, Sawall T, Wiencke C (2001) Light regime in an Arctic fjord: A study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Mar Biol* 138:649–658.

Heesch S, Rindi F, Guiry MD, Nelson WA (2020) Molecular phylogeny and taxonomic reassessment of the genus *Cladostephus* (Sphacelariales, Phaeophyceae). *Eur J Phycol* 55:426–443.

Heinrich S, Valentin K, Frickenhaus S, Wiencke C (2016) Origin matters – Comparative transcriptomics in *Saccharina latissima* (Phaeophyceae). *J Exp Mar Bio Ecol* 476:22–30.

Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed ecology and physiology, Second Edi. Cambridge University Press, Cambridge.

IPCC (2019) IPCC Special report on the ocean and cryosphere in a changing climate. Pörtner H-O, Roberts DC, Masson-Delmotte V, Zhai P, Tignor M, Poloczanska E, Mintenbeck K, Alegría A, Nicolai M, Okem A, Petzold J, Rama B, Weyer NM (eds) Intergovernmental Panel on Climate Change.

Iwamoto K, Shiraiwa Y (2005) Salt-regulated mannitol metabolism in algae. *Mar Biotechnol* 7:407–415.

Ji Y, Xu Z, Zou D, Gao K (2016) Ecophysiological responses of marine macroalgae to climate change factors. *J Appl Phycol* 28:2953–2967.

Jiménez C, Niell FX (1991) Influence of temperature and salinity on carbon and nitrogen content in *Dunaliella viridis* teodoresco under nitrogen sufficiency. *Bioresour Technol* 38:91–94.

Johnston CS, Jones RG, Hunt RD (1977) A seasonal carbon budget for a laminarian population in a Scottish sea-loch. *Helgol wiss Meeresunt* 30:527–545.

de Jong G (2005) Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. *New Phytol* 166:101–118.

Josse J, Husson F (2016) MissMDA: A package for handling missing values in multivariate data analysis. *J Stat Softw* 70:1–31.

Karsten U (2007) Salinity tolerance of Arctic kelps from Spitsbergen. *Phycol Res* 55:257–262.

Karsten U (2012) Seaweed acclimation to salinity and desiccation stress. In: *Seaweed Biology – Novel insights into ecophysiology, ecology and utilization.* Wiencke C, Bischof K (eds) Springer-Verlag Berlin Heidelberg, p 87–107.

Kautsky L, Kautsky N (2000) The Baltic Sea, including Bothnian Sea and Bothnian Bay. In: *Seas at the millennium: An environmental evaluation*. Sheppard CRC (ed) Elsevier Science Ltd.

King NG, Mckeown NJ, Smale DA, Moore PJ (2018) The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. *Ecography* 41:1469–1484.

Kirst GO (1989) Salinity tolerance of eukaryotic marine algae. *Annu Rev Plant Physiol Plant Mol Biol* 40:21–53.

Lane CE, Lindstrom SC, Saunders GW (2007) A molecular assessment of northeast Pacific *Alaria* species (Laminariales, Phaeophyceae) with reference to the utility of DNA barcoding. *Mol Phylogenet Evol* 44:634–648.

Li H, Monteiro C, Heinrich S, Bartsch I, Valentin K, Harms L, Glöckner G, Corre E, Bischof K (2020) Responses of the kelp *Saccharina latissima* (Phaeophyceae) to the warming Arctic: From physiology to transcriptomics. *Physiol Plant* 168:5–26.

Liesner D, Fouqueau L, Valero M, Roleda MY, Pearson GA, Bischof K, Valentin K, Bartsch I (2020) Heat stress responses and population genetics of the kelp *Laminaria digitata* (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations. *Ecol Evol* 10: 9144–9177.

Lourenço CR, Zardi GI, McQuaid CD, Serrão EA, Pearson GA, Jacinto R, Nicastro KR (2016) Upwelling areas as climate change refugia for the distribution and genetic diversity of a marine macroalga. *J Biogeogr* 43:1595–1607.

Lüning K (1990) Seaweeds – Their environment, biogeography, and ecophysiology. Yarish C, Kirkman H (eds) John Wiley & Sons, Inc.

Lüning K (1984) Temperature tolerance and biogeography of seaweeds: The marine algal flora of Helgoland (North Sea) as an example. *Helgol Meeresunt* 28:305–317.

Luttikhuizen PC, van den Heuvel FHM, Rebours C, Witte HJ, van Bleijswijk JDL, Timmermans K (2018) Strong population structure but no equilibrium yet: Genetic connectivity and phylogeography in the kelp *Saccharina latissima* (Laminariales, Phaeophyta). *Ecol Evol* 8:4265–4277.

Mandal SK, Ajay G, Monisha N, Malarvizhi J, Temkar G, Mantri VA (2015) Differential response of varying temperature and salinity regimes on nutrient uptake of drifting fragments of *Kappaphycus alvarezii*: implication on survival and growth. *J Appl Phycol* 27:1571–1581.

Manns D, Nielsen MM, Bruhn A, Saake B, Meyer AS (2017) Compositional variations of brown seaweeds *Laminaria digitata* and *Saccharina latissima* in Danish waters. *J Appl Phycol* 29:1493–1506.

Martins CDL, Lhullier C, Ramlov F, Simonassi JC, Gouvea LP, Noernberg M, Maraschin M, Colepicolo P, Hall-Spencer JM, Horta PA (2014) Seaweed chemical diversity: An additional and efficient tool for coastal evaluation. *J Appl Phycol* 26:2037–2045.

Martins N, Pearson GA, Bernard J, Serrão EA, Bartsch I (2020) Thermal traits for reproduction and recruitment differ between Arctic and Atlantic kelp *Laminaria digitata*. *PLoS One* 15:e0235388.

Monteiro C, Li H, Diehl N, Collén J, Heinrich S, Bischof K, Bartsch I (2021) Modulation of physiological performance by temperature and salinity in the sugar kelp *Saccharina latissima*. *Phycol Res* 69:48–57.

Monteiro JP, Rey F, Melo T, Moreira ASP, Arbona JF, Skjermo J, Forbord S, Funderud J, Raposo D, Kerrison PD, Perrineau MM, Gachon C, Domingues P, Calado R, Domingues MR (2020) The unique lipidomic signatures of *Saccharina latissima* can be used to pinpoint their geographic origin. *Biomolecules* 10:107.

Morel A (1991) Light and marine photosynthesis: a spectral model with geochemical and climatological implications. *Prog Oceanogr* 26:263–306.

Müller J, Müller K, Neinhus C, Quandt D (2010) PhyDE® (Phylogenetic Data Editor). http://www.phyde.de/index.html.

Müller R, Laepple T, Bartsch I, Wiencke C (2009) Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Bot Mar* 52:617–638.

Neiva J, Paulino C, Nielsen MM, Krause-Jensen D, Saunders GW, Assis J, Bárbara I, Tamigneaux É, Gouveia L, Aires T, Marbà N, Bruhn A, Pearson GA, Serraõ EA (2018) Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissima*. *Sci Rep* 8:1112.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. *Trends Plant Sci* 15:684–692.

Nielsen MM, Manns D, D'Este M, Krause-Jensen D, Rasmussen MB, Larsen MM, Alvarado-Morales M, Angelidaki I, Bruhn A (2016a) Variation in biochemical composition of *Saccharina latissima* and *Laminaria digitata* along an estuarine salinity gradient in inner Danish waters. *Algal Res* 13:235–245.

Nielsen MM, Paulino C, Neiva J, Krause-Jensen D, Bruhn A, Serrão EA (2016b) Genetic diversity of *Saccharina latissima* (Phaeophyceae) along a salinity gradient in the North Sea-Baltic Sea transition zone. *J Phycol* 52:523–531.

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annu Rev Ecol Evol Syst* 37:637–671.

Pehlke C, Bartsch I (2008) Changes in depth distribution and biomass of sublittoral seaweeds at Helgoland (North Sea) between 1970 and 2005. *Clim Res* 37:135–147.

Peters KJ, Amsler CD, Amsler MO, McClintock JB, Dunbar RB, Baker BJ (2005) A comparative analysis of the nutritional and elemental composition of macroalgae from the western Antarctic Peninsula. *Phycologia* 44:453–463.

QGIS Development Team (2019) QGIS 3.8.2-Zanzibar software. http://qgis.osgeo.org.

Reed TE, Schindler DE, Waples RS (2011) Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conserv Biol* 25:56–63.

Roleda MY, Hurd CL (2019) Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. *Phycologia* 58:552–562.

Rozema J, Björn LO, Bornman JF, Gaberscik A, Häder DP, Trost T, Germ M, Klisch M, Gröninger A, Sinha RP, Lebert M, He Y-Y, Buffoni-Hall R, de Bakker NVJ, van de Staaji J, Meijkamp BB (2002) The role of UV-B radiation in aquatic and terrestrial ecosystems – an experimental and functional analysis of the evolution of UV-absorbing compounds. *J Photochem Photobiol B Biol* 66:2–12.

Schiener P, Black KD, Stanley MS, Green DH (2015) The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*. *J Appl Phycol* 27:363–373.

Schoenwaelder MEA, Clayton MN (1999) The presence of phenolic compounds in isolated cell walls of brown algae. *Phycologia* 38:161–166.

Simonson EJ, Scheibling RE, Metaxas A (2015) Kelp in hot water: I. Warming seawater temperature induces weakening and loss of kelp tissue. *Mar Ecol Prog Ser* 537:89–104.

Sjøtun K (1993) Seasonal lamina growth in two age groups of *Laminaria saccharina* (L.) Lamour. in Western Norway. *Bot Mar* 36:433–442.

Spurkland T, Iken K (2011) Salinity and irradiance effects on growth and maximum photosynthetic quantum yield in subarctic *Saccharina latissima* (Laminariales, Laminariaceae). *Bot Mar* 54:355–365.

Stévant P, Indergård E, Ólafsdóttir A, Marfaing H, Larssen WE, Fleurence J, Roleda MY, Rustad T, Slizyte R, Nordtvedt TS (2018) Effects of drying on the nutrient content and physico- chemical and sensory characteristics of the edible kelp *Saccharina latissima*. *J Appl Phycol* 30: 2587–2599.

Valero M, Destombe C, Mauger S, Ribout C, Engel CR, Daguin-Thiebaut C, Tellier F (2011) Using genetic tools for sustainable management of kelps: A literature review and the example of *Laminaria digitata*. *Cah Biol Mar* 52:467–483.

Viktorsson L (2017) Hydrography and oxygen in the deep basins. HELCOM program Report.

Vu VQ (2011) Ggbiplot: A ggplot2 based biplot. R package.

Wiencke C, Bischof K (2012) Seaweed Biology – Novel insights into ecophysiology, ecology and utilization. Springer, Heidelberg New York Dodrecht London.

Wiencke C, Vögele B, Kovaltchouk NA, Hop H (2004) Species composition and zonation of marine benthic macroalgae at Hansneset in Kongsfjorden, Svalbard. In: *The coastal ecosystem of Kongsfjorden, Svalbard. Synopsis of biological research performed at the Koldewey Station in the years 1991 – 2003*. Wiencke C (ed) Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, p 55–62.

Supplement

Table S5.1: Overview of the morphology and biochemical parameters of all *Saccharina latissima* samples. Frond length, frond width and length to width ratio (length:width). Mannitol, carbon to nitrogen ratio (C:N), total nitrogen content (total N), total carbon content (total C) and phlorotannins. DW = dry weight.

location			*data-set*	*morphology*			*biochemical parameters*				
				length (cm)	**width (cm)**	**length:width**	**mannitol (mg g^{-1} DW)**	**C:N**	**total N (mg g^{-1} DW)**	**total C (mg g^{-1} DW)**	**phlorotannins (mg g^{-1} DW)**
Ny-Ålesund	NYA	Norway	latitudinal	142	59	2.4	210.96	22.56	14.84	287.02	1.467
				107	40	2.7	196.46	23.85	15.42	315.28	1.705
				110	45	2.4	162.06	21.45	14.35	263.81	2.487
				105	38	2.8	208.98	19.67	15.57	262.64	2.119
				80	44	1.8	208.16	22.09	16.85	319.07	1.980
Tromsø	TRO	Norway	latitudinal	113	25	4.5	123.86	23.20	13.70	272.41	6.019
				130	19	6.8	280.88	37.58	9.71	312.95	3.392
				80	14.5	5.5	102.12	19.41	16.01	266.27	10.184
				115	23	5.0	71.78	17.36	16.97	252.52	4.697
				84	16	5.3	64.24	16.15	15.83	219.15	4.095
Bodø	BOD	Norway	latitudinal	252	36	7.0	183.81	23.95	14.77	303.24	8.493
				309	43	7.2	214.10	27.11	13.39	311.03	9.403
				237	38	6.2	215.27	24.58	13.72	289.19	11.856
				130	26	5.0	230.37	31.78	12.10	329.51	10.170
				110	35	3.1	223.97	29.82	12.75	325.98	7.587
Ansnas	ANS	Norway	latitudinal	54	23	2.3	80.15	-	-	-	-
				73	30	2.4	109.96	23.05	11.40	225.29	6.737
				120	55	2.2	155.68	33.55	8.02	230.65	6.288
				92	42	2.2	190.40	27.82	15.09	359.88	8.193
				140	43	3.3	178.25	29.57	13.35	338.49	6.837
Runde (sandy)	RUN_s	Norway	latitudinal	104	27	3.9	133.98	53.52	7.59	348.06	14.579
				146	25	5.8	173.53	48.98	8.45	354.81	17.677
				258	33	7.8	172.20	33.60	11.09	319.43	18.017
				128	25	5.1	163.02	44.57	8.74	333.95	17.040
				107	28	3.8	169.15	55.12	8.46	399.76	17.800

continues on next page

Runde (rocky)	RUN_r	Nor.	lati-tud.	223	12.5	17.8	278.31	25.66	14.12	310.57	5.514
				147	11.5	12.8	344.64	40.38	10.84	375.29	6.330
				196	9.5	20.6	259.91	34.86	10.19	304.56	5.987
Bergen	BER	Norway	latitudinal	64	23	2.8	169.27	23.09	15.63	309.20	14.730
				113	30	3.8	212.01	24.62	14.90	314.49	7.401
				77	24	3.2	179.69	25.22	16.92	365.81	7.033
				79	28	2.8	140.42	19.29	20.85	344.71	3.621
				103	36	2.9	116.13	21.35	16.64	304.42	9.763
Egersund	EGE	Norway	latitudinal & Baltic	191	35	5.5	163.11	21.19	17.74	322.15	10.834
				109	27	4.0	122.82	27.28	15.43	360.95	13.629
				137	26	5.3	126.11	22.67	17.65	343.01	5.605
				136	34	4.0	97.69	26.52	15.87	360.70	11.078
				125	31	4.0	118.40	16.70	23.13	331.12	9.258
Helgoland	HLG	Germany	latitudinal	345	14.5	23.8	203.27	15.00	-	-	6.238
				205	12.5	16.4	277.14	28.14	-	-	7.355
				167	11.5	14.5	270.38	32.20	10.38	286.59	7.239
				138.5	10.8	12.8	214.35	37.09	10.61	337.37	8.888
				227	15	15.1	240.17	32.12	12.68	349.00	8.634
Locmaria-quer	LOC	France	latitudinal	250	13	18.9	183.66	16.80	19.94	287.13	5.572
				168	25	6.7	225.84	30.07	13.46	346.75	8.231
				139	20	7.0	206.01	24.24	15.10	313.73	6.840
				350	22	15.9	174.23	20.72	16.25	288.54	5.202
				228	31	7.4	211.41	33.25	12.41	353.67	5.927
Muros	MUR	Spain	latitudinal	70	11	6.4	59.00	12.40	25.17	267.48	6.389
				86	13	6.6	200.12	21.82	17.44	326.16	7.460
				75	14	5.4	119.10	14.67	22.43	282.11	11.270
				56	24	2.3	148.12	15.77	24.28	328.32	6.927
				52	11.5	4.5	98.22	12.82	27.69	304.21	3.423
Amorosa	AMO	Portugal	latitudinal	50	4	12.5	56.80	9.97	28.25	241.33	3.464
				42.5	9	4.7	-	11.23	29.71	286.10	4.401
				41.5	7.2	5.8	63.51	10.99	29.14	274.59	4.899
				40	6.5	6.2	149.95	11.98	28.49	292.62	5.582
				36	5	7.2	118.17	10.92	29.76	278.61	8.729

continues on next page

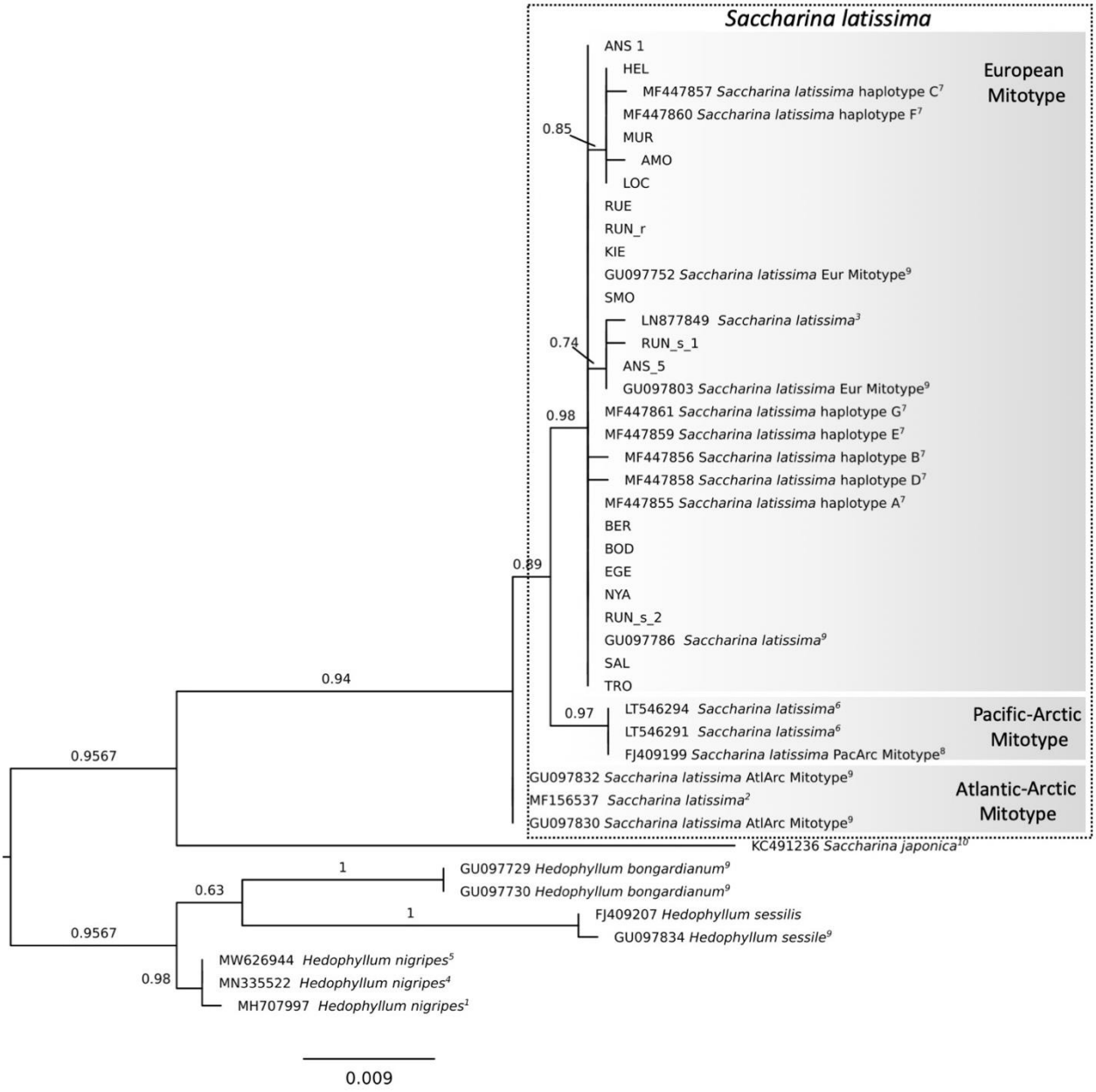
Saltø	SAL	Sweden	Baltic	127	13	9.5	115.56	17.32	23.73	352.41	17.855
				125	17	7.1	102.07	20.71	18.86	334.76	16.775
				140	16	8.8	111.66	23.01	18.39	362.57	10.093
				130	16	8.2	117.51	17.88	21.58	330.66	10.614
				143	14	9.9	144.24	15.95	24.62	336.51	17.506
Smøgen	SMO	Sweden	Baltic	218	18	11.8	119.23	20.76	18.77	334.01	14.361
				294	14	20.9	120.62	22.13	17.84	338.35	16.624
				158	16	9.8	88.02	28.10	15.86	382.07	7.132
				239	18	13.4	152.05	26.44	15.64	354.57	18.925
				166	17	9.6	78.80	25.73	17.28	380.99	10.187
Kiel	KIE	Germany	Baltic	57	10	5.7	119.01	16.12	22.66	313.06	6.948
				47	10	4.8	44.67	11.85	26.72	271.34	3.869
				40	10	3.8	83.28	14.49	24.70	306.87	8.214
				45	6	7.9	61.42	13.37	24.52	281.00	3.182
				42	6	6.8	97.84	14.97	22.97	294.80	7.082
Rügen	RUG	Germany	Baltic	25.5	8.6	3.0	47.67	27.37	14.54	341.08	3.702
				23.5	5	4.7	57.21	23.62	17.84	361.21	5.710
				13	6.4	2.0	77.02	17.60	21.44	323.43	7.663
				5.6	4.1	1.4	92.82	13.82	28.04	332.05	6.831
				5.4	1.1	4.9	-	-	-	-	17.077

Table S5.2: Pearson correlations of morphological and biochemical parameters of European *Saccharina latissima* and abiotic factors: sampling depth, sea surface temperature (SST) and salinity. $p < 0.05$*; $p < 0.01$**; $p < 0.001$***.

data-set	correlated factors	*t*	*df*	*p*	*r*
all	C – mannitol	1.350	72	0.181	0.158
	C – phlorotannins	4.124	72	< 0.001	0.437***
latitudinal	length – SST	1.396	56	0.168	0.183
	width – SST	−4.385	56	< 0.001	−0.506***
	length:width – SST	3.275	56	0.002	0.401**
	length – salinity	−2.442	56	0.018	−0.310*
	width – salinity	−2.947	56	0.005	−0.366**
	length:width – salinity	−0.221	56	0.823	−0.030
	length – depth	1.060	56	0.294	0.140
	width – depth	1.435	56	0.157	0.188
	length:width – depth	0.731	56	0.468	0.097
	mannitol – length	3.629	55	< 0.001	0.440***
	mannitol – width	1.047	55	0.230	0.140
	mannitol – length:width	2.904	55	0.005	0.365**
	C:N – length	2.256	54	0.028	0.293*
	C – length	1.265	53	0.211	0.171
	N – length	−3.610	53	< 0.001	−0.444***
	C:N – width	1.992	54	0.051	0.262
	C – width	0.837	53	0.407	0.114
	N – width	−3.875	53	< 0.001	−0.470***
	C:N – length:width	0.446	54	0.658	0.061
	C – length:width	−0.201	53	0.841	−0.028
	N – length:width	−0.357	53	0.723	−0.049
	phlorotannins – length	0.900	55	0.373	0.120
	phlorotannins – width	0.905	55	0.370	0.121
	phlorotannins – length:width	−1.000	55	0.322	−0.134
	mannitol – SST	−0.580	55	0.564	−0.078
	mannitol – salinity	−2.878	55	0.006	−0.362**
	mannitol – depth	1.994	55	0.051	0.260
	C:N – SST	−0.940	54	0.351	−0.127
	C – SST	2.294	53	0.026	0.301*
	N – SST	2.733	53	0.009	0.351**
	C:N – salinity	−4.786	54	< 0.001	−0.546***
	C – salinity	−1.960	53	0.055	−0.260
	N – salinity	6.870	53	< 0.001	0.686***
	C:N – depth	−1.432	54	0.158	−0.191
	C – depth	0.039	53	0.969	0.005
	N – depth	0.673	53	0.504	0.092
	phlorotannins – SST	0.958	55	0.342	0.128
	phlorotannins – salinity	−1.625	55	0.110	−0.214
	phlorotannins - depth	−2.590	55	0.012	−0.330*
Baltic	length – SST	0.685	23	0.500	0.141
	width – SST	−2.855	23	0.009	−0.512**
	length:width – SST	2.610	23	0.016	0.478*
	length – salinity	3.650	23	0.001	0.606**
	width – salinity	8.751	23	< 0.001	0.877***
	length:width – salinity	0.981	23	0.337	0.200
	length – depth	0.001	23	0.999	0.000
	width – depth	0.009	23	0.993	0.012
	length:width – depth	−0.590	23	0.561	−0.078
	continues on next page				

mannitol – length	4.1806	22	< 0.001	0.665***
mannitol – width	3.4177	22	0.002	0.589**
mannitol – length:width	1.8978	22	0.071	0.375
C:N – length	2.350	22	0.028	0.448*
C – length	2.176	22	0.041	0.421*
N – length	−2.578	22	0.017	−0.482*
C:N – width	2.266	22	0.034	0.435*
C – width	1.740	22	0.096	0.348
N – width	−2.472	22	0.022	−0.466*
C:N – length:width	0.975	22	0.340	0.203
C – length:width	1.00	22	0.328	0.209
N – length:width	−1.158	22	0.260	−0.240
phlorotannins – length	3.338	23	0.003	0.571**
phlorotannins – width	1.050	23	0.305	0.214
phlorotannins – length:width	3.101	23	0.005	0.543**
mannitol – SST	−1.461	22	0.158	−0.297
mannitol – salinity	4.166	22	< 0.001	0.664***
mannitol – depth	0−196	22	0.847	0.042
C:N – SST	0.297	22	0.769	0.063
C – SST	−0.049	22	0.961	−0.010
N – SST	−0.222	22	0.826	−0.222
C:N – salinity	1.029	22	0.315	0.214
C – salinity	1.165	22	0.257	0.241
N – salinity	−1.173	22	0.253	−0.243
C:N – depth	2.741	22	0.012	0.505*
C – depth	3.915	22	< 0.001	0.641***
N – depth	−2.114	22	0.046	−0.411*
phlorotannins – SST	−0.171	23	0.866	−0.036
phlorotannins – salinity	1.695	23	0.104	0.333
phlorotannins – depth	1.192	23	0.246	0.241

Fig. S5.1: Maximum Likelihood phylogram based on COI-5P sequences of the genera *Saccharina* and *Hedophyllum*. Values above nodes indicate ML bootstrap support, with values lower than 60 % not reported. The scale indicates substitutions / site. Published sequences are indicated by their GenBank accession number, the species name, haplotype (where applicable) and a number specifying the reference: 1 - Aizen et al. (unpublished); 2 – Augyte et al. (2018); 3 – Biancarosa et al. (2016); 4 – Bringloe et al. (2020); 5 – Franke et al. (unpublished); 6 – Kuepper et al. (2016); 7 – Luttikhuizen et al. (2018); 8 – McDevit & Saunders (2009); 9 – McDevit & Saunders (2010); 10 – Zhao et al. (2013).



References for Figure S5.1

1 – Aizen V, Armos BM, Hahn JL, Kittle RP, Owen VM, Perez FP, Poulin E, Rocha MA, Rojas-Angulo M, Rouske J, Spencer DP, Tonra KJ, Woodworth KA, Zuelow AN, Wells C, Freshwater DW, Mumford TF (unpublished) MASJI–5: A.L.G.A.E. (Always Looking Good And Enthusiastic).

2 – Augyte S, Lewis L, Lin S, Neefus CD, Yarish C (2018) Speciation in the exposed intertidal zone: the case of *Saccharina angustissima* comb. nov. & stat. nov. (Laminariales, Phaeophyceae). *Phycologia* 57:100–112.

3 – Biancarosa I, Espe M, Bruckner CG, Heesch S, Liland NS, Waagbø R, Torstensen B, Lock EJ, (2016) Amino acid composition, protein content, and nitrogen-to-protein conversion factors of 21 seaweed species from Norwegian waters. *J Appl Phycol* 29:1001–1009.

4 – Bringloe TT, Verbruggen H, Saunders GW (2020) Unique biodiversity in Arctic marine forests is shaped by diverse recolonization pathways and far northern glacial refugia. *Proc Natl Acad Sci U S A* 117:22590–22596.

5 – Franke K, Liesner D, Heesch S, Bartsch I (in revision) Temperature performance of Arctic kelps *Laminaria digitata* and *Hedophyllum nigripes* (Laminariales, Phaeophyceae) reveal contrasting growth, reproduction and survival ranges. *Bot Mar* (in revision).

6 – Kuepper FC, Peters AF, Shewring DM, Sayer MD, Mystikou A, Brown H, Azzopardi E, Dargent O, Strittmatter M, Brennan D, Asensi AO, Van West P, Wilce RT (2016) Arctic marine phytobenthos of northern Baffin Island. *J Phycol* 52:532–549.

7 – Luttikhuizen PC, van den Heuvel FHM, Rebours C, Witte HJ, van Bleijswijk JDL, Timmermans K (2018) Strong population structure but no equilibrium yet: Genetic connectivity and phylogeography in the kelp *Saccharina latissima* (Laminariales, Phaeophyta). *Ecol Evol* 8:4265–4277.

8 – McDevit DC, Saunders GW (2009) On the utility of DNA barcoding for species differentiation among brown macroalgae (Phaeophyceae) including a novel extraction protocol. *Phycol Res* 57:131–141.

9 – McDevit DC, Saunders GW (2010) A DNA barcode examination of the Laminariaceae (Phaeophyceae) in Canada reveals novel biogeographical and evolutionary insights. *Phycologia* 49:235–248.

10 – Zhao X, Pang S, Shan T, Liu F (2013) Applications of three DNA barcodes in assorting intertidal red macroalgal flora in Qingdao, China. *J Ocean Univ China* 12:139–145.

Fig. S5.2: Combined score- & biplot of the principal component analyses (PCA) performed on samples of *Saccharina latissima* from different sample locations along the European coast. Plotted are frond length and width. The arrows represent the loadings of each PCA. Sampling locations are indicated by different colors. Different shapes represent hydrographic regions of the samples: ♦ = Arctic (Spitsbergen), ▲ = Norwegian Sea, ■ = North Sea / North Atlantic, ● = Baltic Sea. Each point represents one individual.

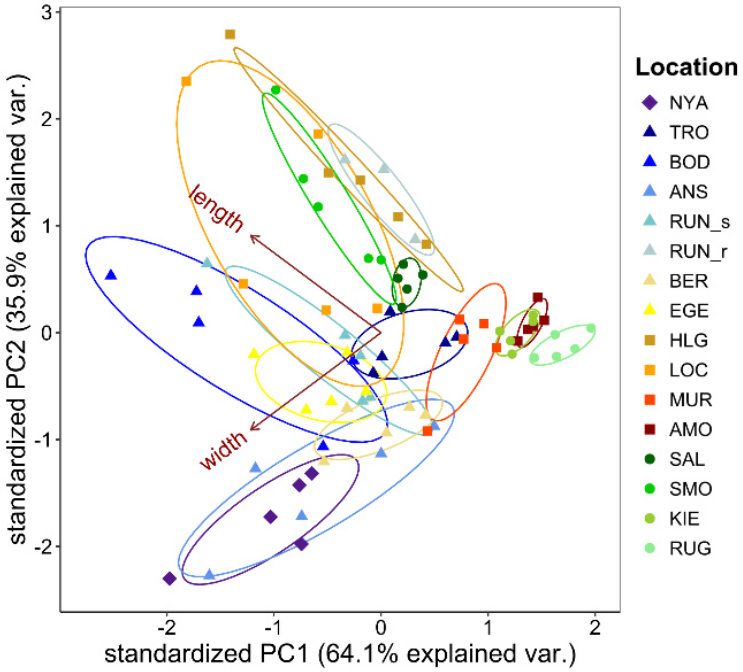


Fig. S5.3: Combined score- & biplot of the principal component analyses (PCA) performed on samples of *Saccharina latissima* from different sample locations along the European coast. Plotted are the biochemical compounds (mannitol, carbon to nitrogen ratio (C:N), phlorotannins). The arrows represent the loadings of each PCA. Sampling locations are indicated by different colors. Different shapes represent hydrographic regions of the samples: ♦ = Arctic (Spitsbergen), ▲ = Norwegian Sea, ■ = North Sea / North Atlantic, ● = Baltic Sea. Each point represents one individual.

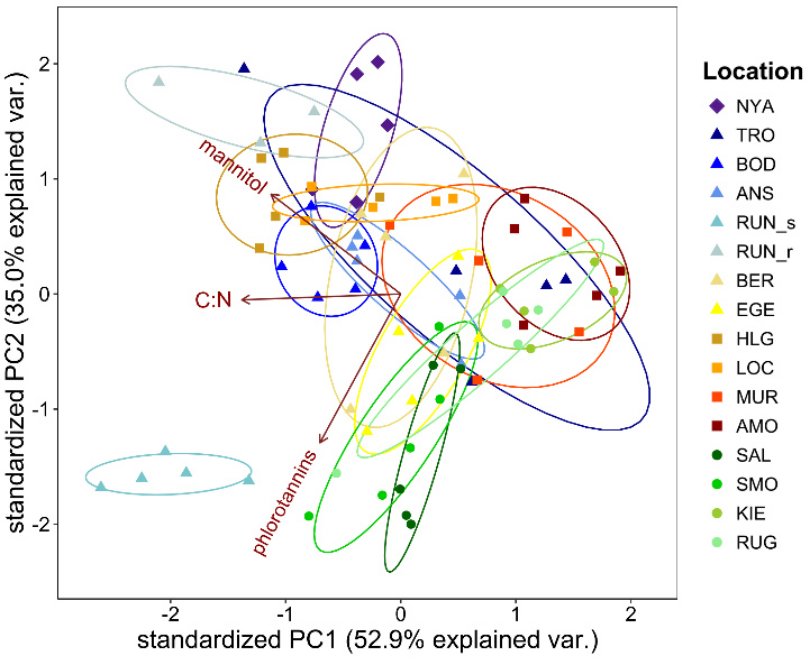
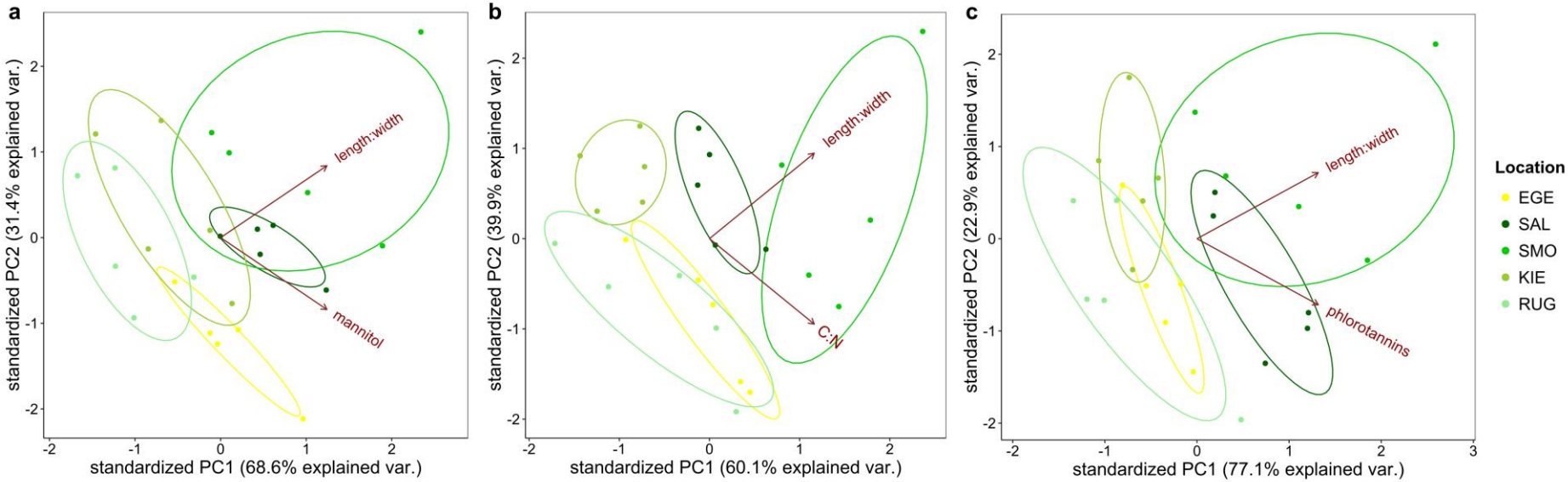


Fig. S5.4: Combined score- & biplot of the principal component analyses (PCA) performed on samples of *Saccharina latissima* from different sample locations along a salinity gradient. Plotted are the length to width ratio (length:width) and the biochemical compounds individually. **a**: Mannitol. **b**: Carbon to nitrogen ratio (C:N). **c**: Phlorotannins. The arrows represent the loadings of each PCA. Sampling locations are indicated by different colors. Each point represents one individual.



6. Publication V:

Impacts of combined temperature and salinity stress on the endemic Arctic brown seaweed *Laminaria solidungula* J. Agardh

Nora Diehl, Ulf Karsten, Kai Bischof

published in
Polar Biology
April 2020

doi: 10.1007/s00300-020-02668-5

Title: Impacts of combined temperature and salinity stress on the endemic Arctic brown seaweed *Laminaria solidungula* J. Agardh

Authors: Nora Diehl, Ulf Karsten, Kai Bischof

Abstract

Macroalgae such as kelp are important ecosystem engineers in the polar regions and potentially affected by freshening and ocean warming. The endemic Arctic kelp *Laminaria solidungula* might be particularly imperiled and become locally extinct from Arctic fjord systems in the future, since temperature increase is most pronounced in the polar regions. Additionally, increased temperatures cause glacier and sea ice melting and enhancing terrestrial run-off from snowfields, which eventually can result in hyposaline conditions in fjord systems. We conducted a multiple-stressor experiment at four temperatures (0, 5, 10, 15 °C) and two salinities (S_A 25, 35) to investigate the combined effects of increasing temperature and decreasing salinities on the physiological and biochemical status of young *L. solidungula* sporophytes. Both drivers had significant and interacting impacts, either in an additive or antagonistic way, dependent on the respective response variable. The maximum quantum yield of photosystem II (F_v/F_m) significantly declined with temperature increase and low salinity. Even though the absolute pigment content was not affected, the de-epoxidation state of the xanthophyll cycle increased with intensified stress. Higher temperatures affected the C:N ratio significantly, mainly due to reduced nitrogen uptake, while S_A 25 supported the nitrogen uptake, resulting in an attenuation of the effect. The concentration of mannitol decreased at S_A 25. At control S_A 35 mannitol level remained steady between 0 and 10 °C but significantly decreased at 15 °C. Conclusively, our results show that *L. solidungula* is very susceptible to both drivers of climate change, especially when they are combined. Implications to species ecology are discussed.

Introduction

Temperature increase is one of the major drivers of climate change. The strongest regional warming over the last 30 years was detected in the polar regions (Maturilli et al. 2013, Meredith et al. 2019). Elevated air and seawater temperatures cause glacier and sea ice melting and increased terrestrial run-off from snowfields (Sundfjord et al. 2017, Filbee-Dexter et al. 2019). Consequently, increased temperatures may result in hyposaline conditions in Arctic fjords, due to limited seawater exchange and stratification (Svendsen et al. 2002). Between 1936 and 1999 total fresh water inflow into the Arctic Ocean increased by about 7 % (Bluhm & Gradinger 2008). Under regular conditions, seawater salinity in Arctic fjords averages S_A 34.5 in spring, and can drop below S_A 23 at the sea surface (Karsten et al. 2003, Zacher et al. 2009). However, salinity in deeper water layers down to 20 m may also be affected after vertical mixing by wave and wind action (Hanelt et al. 2001). Average seawater temperatures in Kongsfjorden, Svalbard are 4 °C during summer, but can reach a maximum of 5 – 7 °C (Hanelt et al. 2001, Svendsen et al. 2002, Bartsch et al. 2016). In 2019, these maxima were exceeded reaching almost 8 °C, which demonstrates the regional fast increases of seawater temperature (unpublished, Dashboard AWI 2019). In other Arctic regions, the sea surface temperature has already increased up to 8 – 10 °C (Wiencke et al. 2007). Long-term trends for the Arctic region predict a further increase in warming and reduction of sea ice cover (Müller et al. 2009, Walczowski et al. 2012, Oliver et al. 2018), and increasing temperatures, especially heatwave events, are likely to impact many marine species (Smale et al. 2019).

Arctic macroalgae are specifically affected by freshening and ocean warming (Hanelt et al. 2001). More than 140 seaweed species in the Arctic region have been described so far, most of them growing in the sublittoral (Hop et al. 2012). Kelps, large brown seaweeds of the order Laminariales, are important ecosystem engineers in Arctic fjords. The only endemic kelp species from the Arctic is *Laminaria solidungula* J. Agardh, which can be found down to depths of 18 m (Roleda 2016). It grows at temperatures up to 16 °C with an optimum at 5 – 10 °C (tom Dieck [Bartsch] 1992). Despite the general ability of seaweeds to acclimate to variation in temperature and other environmental drivers (e.g. Davison et al. 1991, Bischof et al. 2002, Graiff et al. 2015b, Diehl et al. 2019), there is growing concern that *L. solidungula* might become locally extinct from Arctic fjord systems in the future (Müller et al. 2009). Temperature stress can result in structural weakening of kelp tissue (Simonson et al. 2015b) and has an influence on carbon and nitrogen content, due to structure and nitrogen storage (Atkinson &

Smith 1983, Peters et al. 2005). It also affects photosynthetic quantum yield (F_v/F_m) and pigment concentrations (Andersen et al. 2013, Fernandes et al. 2016). Various, potentially interacting, drivers may additionally influence kelp fitness and competitive success. In order to cope with variation in environmental drivers, such as salinity or irradiation, seaweeds developed different acclimation mechanisms to maintain cellular functions (e.g. Karsten & West 2000, Eggert et al. 2007, Rautenberger et al. 2015, Ji et al. 2016). Among others, brown algae synthesize the polyol mannitol to compensate osmotic stress (Iwamoto & Shiraiwa 2005). Mannitol acts as a compatible solute, by conserving intracellular homeostasis and potentially functioning as antioxidant (Kirst 1989, Iwamoto & Shiraiwa 2005, Eggert et al. 2007). Furthermore, it is known that salinity changes substantially affect nitrogen metabolism in seaweed (Gordillo et al. 2002, Mandal et al. 2015).

Consequently, kelp forests have already been directly and indirectly impaired by large-scale environmental change (Müller et al. 2009, Bartsch et al. 2016). Until today most studies on environmental impacts on seaweeds were designed as uni-factorial experiments (e.g. Bischof 2002, Karsten 2007, Wiencke et al. 2007, Olischläger et al. 2012, Simonson et al. 2015a) or studied interactions between irradiation or acidification and temperature (e.g. Fredersdorf et al. 2009, Heinrich et al. 2015, Gordillo et al. 2016, Springer et al. 2017).

While the effects of irradiance and temperature on kelps have been studied in detail, little is known on salinity tolerance of polar seaweeds (e.g. Karsten et al. 1991a, b, Jacob et al. 1991, 1992, Karsten 2007, Li et al. 2019), and even less on salinity temperature interactions (Russell 1987, Thomas et al. 1988, Fredersdorf et al. 2009, Mandal et al. 2015), especially with regard to the endemic kelp *L. solidungula*.

The present study reveals physiological and biochemical responses to increasing temperature and changing salinities for the hitherto understudied Arctic endemic kelp species *L. solidungula.* Results obtained shed light on the adaptive responses of the species to predict kelp performance in a continuously changing Arctic environment.

Material and methods

Laminaria solidungula *cultivation*

A gametophyte strain of *Laminaria solidungula* (as stock culture obtained from the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, AWI culture number 3130 – not separated into male and female gametophytes) from Spitsbergen was fragmented into multicellular gametophyte filaments and subjected to 0 °C, short-day conditions (4:20-h-light:dark [LD]) for induction of fertility (tom Dieck 1989). After a month, fragments were transferred into long-day conditions (16:8-h-LD). The first sporophytes became visible after two months, and were transferred into larger aerated glassware during growth. The size of the glassware was adapted to the size of sporophytes. After five months, the sporophytes were transferred to 5 °C 16:8-h-LD conditions to promote further growth. The sporophytes were cultivated at a photon fluence rate of 30 µmol photons m^{-2} s^{-1} (ProfiLux 3 with LED Mitras daylight 150, GHL Advanced Technology, Kaiserslautern, Germany). The sporophytes were initially cultivated with full Provasoli-enriched seawater (PES, sterile seawater from the North Sea) and after another five months onwards in ½ PES (with Hepes buffer, respectively). The medium was changed every 1 – 2 weeks. One month later, the sporophytes were once again transferred into 0 °C to delay growth until the experiment was conducted six weeks later.

Two-factorial stress experiment: temperature and salinity

Young sporophytes of *Laminaria solidungula* were maintained in aerated 2-L Kautex bottles filled with ½ PES at an artificial photon fluence rate of 30 µmol m^{-2} s^{-1} and a 16:8-h-LD (ProfiLux 3 with LED Mitras daylight 150, GHL Advanced Technology, Kaiserslautern, Germany). The medium was changed twice a week. The samples were acclimated to four different temperatures (0, 5, 10, 15 °C) within one week. The salinity treatments (S_A 25 and 35) were started after temperature acclimation. The hyposaline conditions were maintained by diluting the sterile seawater (North Sea) with freshwater (tap water). The treatment 0 °C, S_A 35 was used as control. Four sporophytes were cultivated as replicates (n = 4) per treatment. After being maintained at treatment conditions for 14 days, all samples were shock-frozen in liquid N_2, stored at −80 °C, and freeze-dried before biochemical analyses.

Response variables

Photo-ecophysiological markers

The photosynthetic performance of the meristem was determined every fourth day by measuring the *in vivo* chlorophyll fluorescence of photosystem II (PS II) using an Imaging-PAM (Walz GmbH Mess- und Regeltechnik, Effeltrich, Germany), after 5 min of dark acclimation. The PAM was set up to determine the amplitude of the fluorescence signal (F_t) between 0.15 and 0.2 as recommended in the manual (IMAGING-PAM M-Series Chlorophyll Fluorometer, Heinz Walz GmbH, Effeltrich, Germany). The maximum quantum yield of PS II (F_v/F_m) represents a sensitive indicator of photosynthetic performance and, hence, of algal fitness, which might be affected by stress exposure (Kirst 1989, Nitschke et al. 2014, Ji et al. 2016).

Photosynthetic and accessory pigments (n = 4) were extracted and analyzed following exactly Koch et al. (2015). Therefore, 0.05 – 0.1 g freshly freeze-dried samples were extracted in 1 mL acetone (90 %, v/v), incubated at 4 °C for 24 h in darkness and analyzed with a High Performance Liquid Chromatography (HPLC). The concentrations of chlorophyll *a* and *c*2 (Chl *a*, Chl *c*2), fucoxanthin (Fuc), β-carotene (β-Car) as well as the pool size of the xanthophyll cycle (VAZ = violaxanthin, antheraxanthin, zeaxanthin) were calculated as µg g^{-1} dry weight (DW). The de-epoxidation state (DPS) of the xanthophyll cycle was calculated after Colombo-Pallotta et al. (2006).

Additionally, the antioxidant phlorotannin was determined after Springer et al. (2017) using the Folin-Ciocalteu method described in Cruces et al. (2012). 20 mg of freeze-dried sample (n = 4) was extracted in 1 mL acetone (70 %, v/v) and incubated for 24 h at 4 °C in darkness. For the analyses, the absorption at λ 730 nm of three aliquots per replicate was determined in a microplate photometer. The total soluble phlorotannin concentration was expressed in mg g^{-1} DW.

Mannitol

1 mL aqueous ethanol (70 %, v/v) was added to three aliquots of 15 – 20 mg of each lyophilized and homogenized sample and incubated in a water bath at 70 °C for 3 – 4 h (n = 4). The vials were vortexed occasionally to keep the samples dispersed. Initially, samples were centrifuged (5 min; 13,000 rpm) and 800 µL of the supernatant was transferred to a fresh vial and evaporated to dryness (Alpha 1–4 LSCplus and RVC 2–5 CDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The pellets were re-dissolved by vortexing and ultrasonic treatments in 800 µL HPLC grade water. The samples were then

centrifuged for another 5 min (13,000 rpm). The obtained supernatant was analyzed using the method of Karsten et al. (1991a) in a HPLC Agilent Technologies system (1200 Series, Santa Clara, California, USA) with an Aminex Fast Carbohydrate Analysis Column HPAP (100 × 7.8 mm, 9 µm, BioRad, Munich, Germany), protected by a guard cartridge (Phenomenex, Carbo-Pb-2 + 4 × 3.00 mm i.d., Aschaffenburg, Germany) with 100 % dH_2O as a mobile phase. The flow rate was adjusted to 1 mL min^{-1} at 10 – 100 bar and 70 °C. For the calibration 0.5, 1.0, 2.5, 5.0 and 10.0 mM D(-)-mannitol standards ($C_6H_{14}O_6$, Roth) were used. Absorption peaks were detected via RI-Detector (35 °C) and analyzed using the software 'ChemStation for LC 3D systems' (Agilent Technologies, Waldbronn, Germany). Mannitol contents were calculated in µmol g^{-1} dry weight (DW).

Carbon, nitrogen and C:N ratio

Total carbon (C) and total nitrogen (N) concentrations as well as C:N ratios were analyzed following Graiff et al. (2015a). Three aliquots of 2 mg (n = 4) of lyophilized and ground samples were weighed into tin cartridges (6 × 6 × 12 mm) and combusted at 950 °C. Acetanilide (C_8H_9NO) was used as standard (Verardo et al. 1990). The contents of C and N were quantified automatically in an elemental analyzer (Vario EL III, Elementar, Langenselbold, Germany). Total C and total N contents were expressed in mg g^{-1} dry weight (DW).

Statistical analysis

All data-sets were tested for normal distribution (Shapiro-Wilk test; $p > 0.05$) and for homogeneity (Levene's test; $p > 0.05$). Data were transformed if needed. Afterwards, two-way ANOVAs were performed for each parameter ($p < 0.05$) and a post hoc Tukey's test applied to reveal significant differences ($p < 0.05$). The pigment data-sets were analyzed using the generalized linear model (GLM) ($p < 0.05$). The statistical analyses were run using RStudio (Version 1.1.383, Boston, MA, USA) and Excel 2016 (Windows, Microsoft Corporation, Redmond, WA, USA).

Results

Photo-ecophysiology

After 14 days, photosynthetic maximum quantum yield (F_v/F_m) showed a significant decrease at 15 °C in both salinities and was significantly lower than the control treatment (F_3 = 62.866, p < 0.0001). In all other treatments the maximum values of 0.684 ± 0.012 (n = 4) were maintained, independent of temperature or salinity. After 7 days of temperature acclimation, F_v/F_m differed between the four temperatures (0.595 ± 0.015 – 0.678 ± 0.010, control: 0.616 ± 0.017; n = 4) but did not significantly change with temperature and salinity stress during the first 11 days of the experiment (**Fig. 6.1**). The low salinity treatment at 15 °C impaired F_v/F_m significantly stronger than the control salinity treatment (S_A 25: 0.396 ± 0.128, S_A 35: 0.533 ± 0.034; n = 4; p = 0.0001) (**Fig. 6.1**).

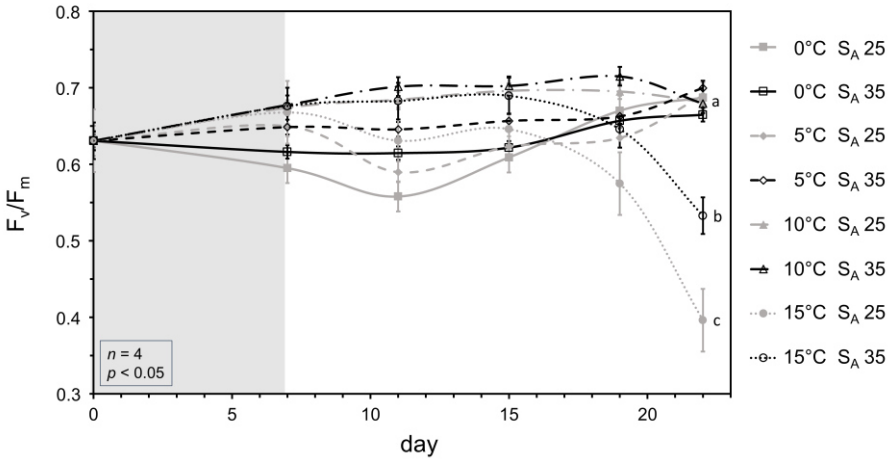


Fig. 6.1: Maximum quantum yield (F_v/F_m) of *Laminaria solidungula* during the multiple-stressor experiment with four temperatures (0, 5, 10, 15 °C) and two salinities (S_A 25, 35). The gray background represents the temperature acclimation phase. The white background represents the temperature × salinity treatment phase. Values are means ± SD (n = 4). Significant differences in final data points are marked with different letters (two-way ANOVA with post hoc Tukey's test; p < 0.05).

Pigment concentrations (GLM: $p < 0.05$, details: **supplement Table S6.1**) and phlorotannin concentrations (temperature: $F_3 = 11.115$, $p < 0.0001$; salinity: $F_1 = 5.884$, $p = 0.0232$; interaction: $F_3 = 3.078$, $p = 0.0467$) exhibited few significant differences, which, however, could not be assigned to any of the stress treatments (**Table 6.1**). Nevertheless, the de-epoxidation state of the xanthophyll cycle (DPS) was significantly affected by temperature ($F_3 = 12.087$, $p < 0.0001$). At 15 °C, DPS is higher than at the other temperature treatments (**Fig. 6.2**). Even tough, there is no strong statistical significance in salinity ($F_1 = 4.478$, $p = 0.0449$), a clear trend to lower DPS values at S_A 35 can be projected. Hence, DPS is increasing with increasing physical stressors.

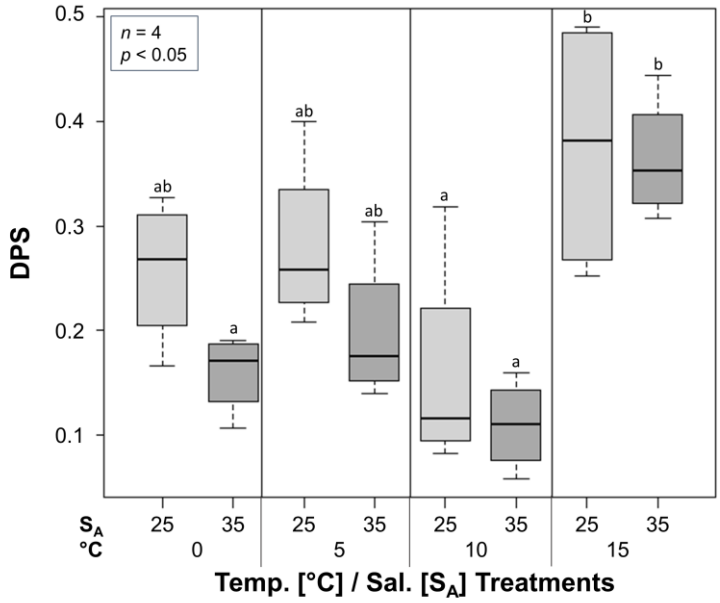


Fig. 6.2: De-epoxidation state of the xanthophyll cycle (DPS) of *Laminaria solidungula* after a two-week exposure in a multiple-stressor experiment with four temperatures (0, 5, 10, 15 °C) and two salinities (S_A 25, 35). Significant differences in final data points are marked with different letters ($n = 4$; two-way ANOVA with post hoc Tukey's test; $p < 0.05$).

Table 6.1: Difference in pigment concentration (µg g^{-1} dry weight [DW]) and phlorotannin concentration (mg g^{-1} DW) in *Laminaria solidungula* after a two-week exposure in a multiple-stressor experiment with four temperatures (0, 5, 10, 15 °C) and two salinities (S_A 25, 35): chlorophyll a (Chl *a*), chlorophyll *c*2 (Chl *c*2), fucoxanthin (Fuc), β-carotene (β-Car), and the pool of the xanthophyll cycle pigments (VAZ: violaxanthin, antheraxanthin, zeaxanthin) and soluble phlorotannins. Significant differences are marked with different letters (n = 4; generalized linear model [GLM]; $p < 0.05$; further statistical details in supplements **Table S6.1**).

Temp. (°C)	S_A	Chl *a*[a] (µg g^{-1} DW)		Chl *c*2 (µg g^{-1} DW)		Fuc[a] (µg g^{-1} DW)		ß-Car[a] (µg g^{-1} DW)		VAZ[b] (µg g^{-1} DW)		Phlorotannins (mg g^{-1} DW)	
0	**25**	602.14 (± 94.29)	a	182.84 (± 11.90)	a	416.49 (± 58.40)	a	77.37 (± 6.04)	a	84.99 (± 52.78)	c	1.29 (± 0.16)	ab
	35	509.75 (± 181.89)	abc	156.37 (± 42.05)	a	352.31 (± 111.02)	a	63.54 (± 13.38)	ab	88.14 (± 6.07)	bc	1.42 (± 0.12)	abc
5	**25**	551.13 (± 67.98)	ab	171.86 (± 13.64)	a	395.16 (± 43.84)	a	75.35 (± 5.13)	ab	75.50 (± 19.56)	abc	1.60 (± 0.19)	c
	35	512.39 (± 77.36)	abc	165.26 (± 10.96)	a	370.47 (± 47.86)	a	71.17 (± 2.42)	ab	55.38 (± 10.24)	ab	1.43 (± 0.15)	cb
10	**25**	406.49 (± 55.12)	bc	128.88 (± 21.67)	a	303.61 (± 41.94)	a	59.27 (± 11.40)	ab	45.36 (± 17.94)	a	1.42 (± 0.13)	ab
	35	878.25 (± 206.58)	c	202.54 (± 22.19)	a	573.65 (± 107.87)	a	77.99 (± 7.86)	ab	85.86 (± 26.29)	c	1.24 (± 0.12)	a
15	**25**	761.35 (± 45.55)	bc	171.24 (± 9.59)	a	517.91 (± 29.52)	a	75.20 (± 4.23)	ab	101.24 (± 6.41)	c	1.35 (± 0.19)	ab
	35	609.79 (± 55.48)	abc	170.94 (± 4.15)	a	418.52 (± 36.11)	a	78.03 (± 2.03)	b	92.34 (± 10.72)	c	1.20 (± 0.18)	a

[a] reciprocal [b] $\log_{10}$ transformation

Mannitol

Temperature and salinity stress affected the mannitol concentration significantly (**Fig. 6.3**), both individually (temperature: $F_3 = 7.038$, $p = 0.0015$; salinity: $F_1 = 79.520$, $p < 0.0001$) and interactively ($F_3 = 79.520$, $p = 0.0027$). Regarding the control salinity treatment (S_A 35), only the 15 °C treatment showed a significant decrease in mannitol from about 1,600 (0 – 10 °C) to 1,323 ± 78 µmol mg^{-1} DW ($n = 4$; 0 °C: $p = 0.0111$; 5 °C: $p = 0.0393$; 10 °C: $p = 0.0266$). The samples incubated at low salinity (S_A 25) generally contained less mannitol than the control salinity samples across all temperatures, with significant differences at 0 °C ($p < 0.0001$) and 5 °C ($p = 0.0002$). Temperature and salinity stress showed an interactive and additive effect on mannitol, since the concentration in samples at S_A 25 increased from 0 °C to 10 °C. Apparently, temperatures beyond the optimum range limited the alga´s ability to compensate salinity differences, resulting in a decrease of mannitol at S_A 35 as well as S_A 25.

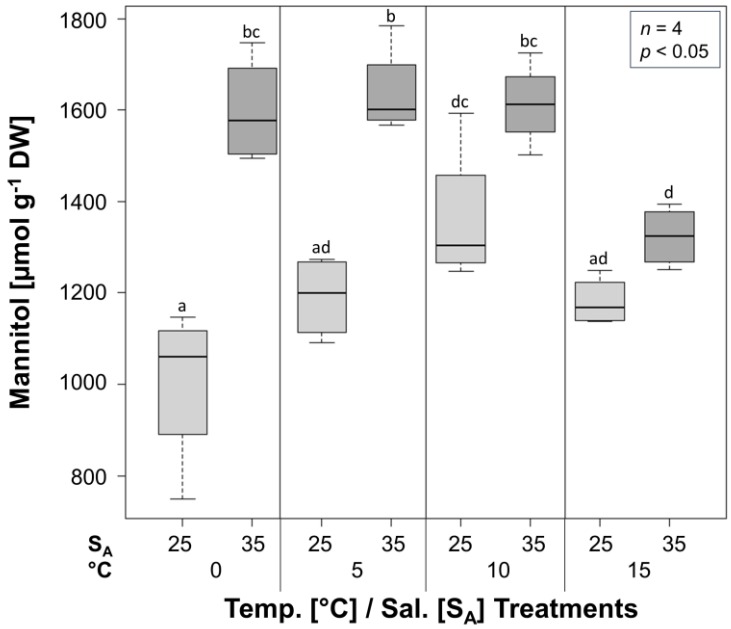


Fig. 6.3: Mannitol concentration (µmol g^{-1} dry weight [DW]) in *Laminaria solidungula* after a two-week exposure in a multiple-stressor experiment with four temperatures (0, 5, 10, 15 °C) and two salinities (S_A 25, 35). Significant differences are marked with different letters ($n = 4$; two-way ANOVA with post hoc Tukey's test; $p < 0.05$).

Carbon, nitrogen and C:N ratio

The carbon:nitrogen (C:N) ratio (**Fig. 6.4a**) was significantly affected by temperature (F_3 = 280.872, p < 0.0001), salinity (F_1 = 121.773, p < 0.0001), and by the interaction of both physical factors (F_3 = 4.025, p = 0.0188). The results indicated an antagonistic effect of increased temperature and low salinity. With higher temperatures, the C:N ratio was significantly higher. The C:N ratio increased above 20 at 10 and 15 °C at both salinities, while it was below 20 at 5 and 10 °C. Additionally, at each temperature, the C:N ratio at S_A 25 was significantly lower than at the control of S_A 35.

For further exploration, the total C and total N content were analyzed. Temperature increase (F_3 = 22.494, p < 0.0001) and salinity decrease (F_1 = 6.564, p = 0.0171) had each a significant and an interactive antagonistic (F_3 = 4.341, p = 0.0140) impact on the total N content (**Fig. 6.4c**). On the one hand, the total N content decreased at higher temperatures (10, 15 °C) at both salinities. On the other hand, the N concentration increased at S_A 25 at each temperature, compared to the control S_A 35. The total C content (**Fig. 6.4b**) barley changed (temperature: F_3 = 178.729, p < 0.0001, salinity: F_1 = 78.823, p < 0.0001), with slight changes in the range of 331 ± 14 and 378 ± 2 mg g^{-1} DW (n = 4). Hence, the increasing C:N ratio was mainly driven by N variations. However, the C content increased at 10 °C/S_A 35, 15 °C/S_A 35 and 15 °C/S_A 25, which balanced out the variation in C:N ratio in these treatments.

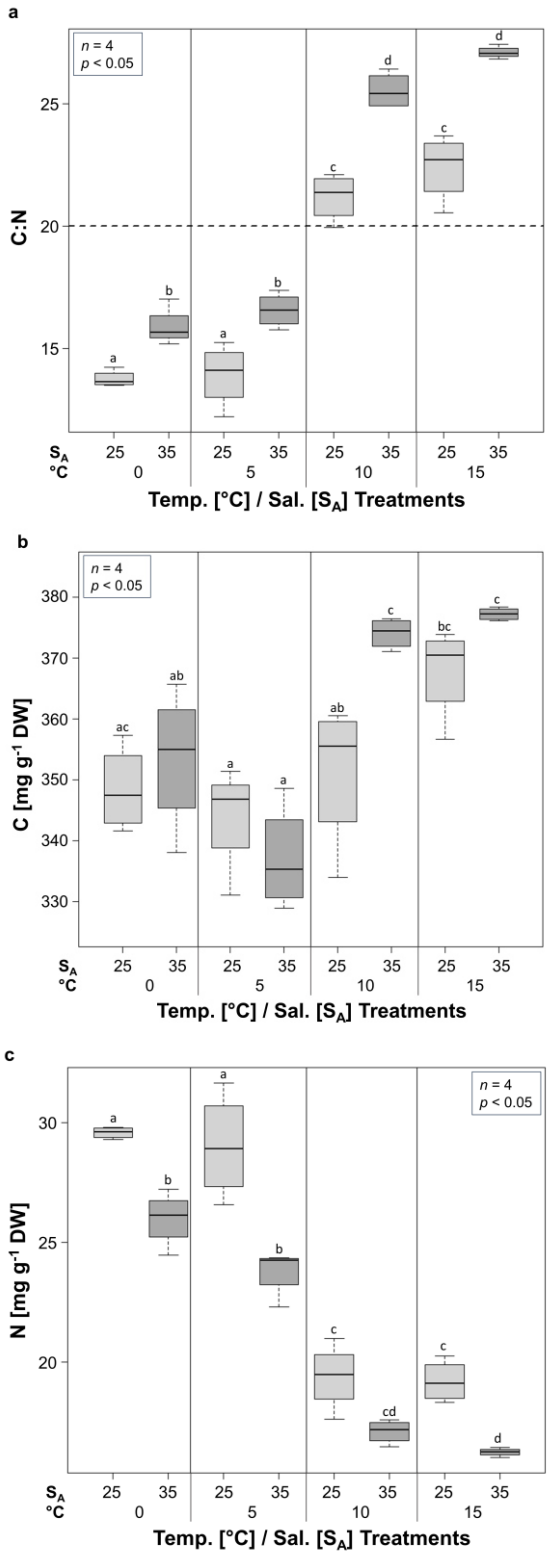


Fig. 6.4: Carbon (C) and nitrogen (N) content in *Laminaria solidungula* after a two-week exposure in a multiple-stressor experiment with four temperatures (0, 5, 10, 15 °C) and two salinities (S_A 25, 35). Significant differences are marked with different letters (n = 4; two-way ANOVA with post hoc Tukey's test; $p < 0.05$). **a:** C:N ratio. The dashed line represents the mean C:N ratio of temperate and tropical seaweeds (Atkinson & Smith 1983). **b:** Total carbon (C) concentration (mg g^{-1} dry weight [DW]). **c:** Total nitrogen (N) concentration [mg g^{-1} DW].

Discussion

The interactive effects of different temperatures and salinities revealed strong physiological and biochemical responses in *Laminaria solidungula* towards a changing environment. It has been shown in macroalgae before that under multiple-stressor conditions, the effects on the physiological performance and biochemical constituents often either interact in an additive or antagonistic manner (Fredersdorf et al. 2009, Gordillo et al. 2016, Springer et al. 2017). As an Arctic endemic species, *L. solidungula* is adapted to low temperatures (tom Dieck [Bartsch] 1992) and it is characterized as a stenohaline species (Karsten 2007). The present study indeed demonstrated the vulnerability of this species to changes in environmental parameters by exposing it to abiotic conditions beyond the ambient range.

As had been observed before, photosynthetic optimum quantum yield (F_v/F_m) was inhibited by lower salinities and temperature stress (Kirst 1989, Fredersdorf 2009, Nitschke et al. 2014, Ji et al. 2016). Independently of salinity, 15 °C has a major impact on F_v/F_m, resulting in a significant diminishment of the photosynthetic capacity. Temperature-induced stress in the thalli of *L. solidungula* was also reported by Parages et al. (2013). Mitogen-activated protein (MAP) kinase-like proteins, which are involved in stress responses, were rapidly activated at 7 °C. Karsten (2007) hypothesized a temperature-limited physiological capacity of cold-temperate and Arctic species to acclimate to external salinity changes. This hypothesis could be confirmed in this study. The combination of low salinity and for the Arctic extreme temperatures of 15 °C inhibited the photosynthetic activity and diminished the mannitol content of *L. solidungula* significantly. Karsten (2007) showed that the photosynthetic performance can be maintained for a short period of stress, but is affected after continued stress exposure, by comparing quantum yields in several Arctic macroalgae after two and five days of treatment. We were able to confirm this effect, however, the significant decrease of F_v/F_m only occurred after two weeks. This shows that *L. solidungula* can compensate stress for a short period but is susceptible if environmental stressors are applied for a longer period. Mannitol is a carbohydrate of low molecular weight and known to play a significant role in the water balance of algal cells (Kirst 1989). Acting as an osmolyte and compatible solute, it preserves the functions of the cells during osmotic stress (Kirst 1989, Eggert et al. 2007). In our experiment, less mannitol was apparently stored in the cells of *L. solidungula* to prevent water flow into the cells under hypoosmotic stress (S_A 25), while at S_A 35 the mannitol content remained almost unchanged. Nevertheless, in this study an impact of temperature was also observed. Since mannitol is the main photosynthetic product of brown algae, it was affected by temperature

(Ji et al. 2016). The optimum growth temperature of *L. solidungula* was determined to be 5 – 10 °C (tom Dieck [Bartsch] 1992). It is generally known that with increasing temperature the enzymatic processes accelerate, while temperature stress above a certain threshold leads to inhibition of metabolic processes (Graiff et al. 2015b). Accordingly, the mannitol concentration in *L. solidungula* increased at S_A 25 between 0 and 10 °C and decreased at 15 °C, likely being in the range of inhibited metabolic processes.

In contrast to studies by Davison et al. (1991) and Celis-Plá et al. (2014), a dependence of the absolute pigment content on growth temperatures, photosynthetic efficiency or other environmental stressors could not be confirmed in this study (**Table 6.1**). Furthermore, contrary to Mannino et al. (2016) and Springer et al. (2017), there was no significant increase or decrease of antioxidants due to stress treatment observed on any level (**Table 6.1**).

Even though no changes in the pool size of xanthophylls (VAZ) were detected, the de-epoxidation state (DPS) was significantly affected by temperature and increased at 15 °C. Furthermore, higher DPS in the low salinity treatments was detected. Changes in the xanthophyll cycle and hence the DPS are an important stress response in seaweeds, mainly as protection against photo-oxidative stress (Müller et al. 2001, Goss & Jakob 2010). Li et al. (2019) and Olischläger et al. (2017) detected an increase of DPS at the suboptimal temperatures in *Saccharina latissima* from the Arctic and Helgoland. These two studies and the result of our study support the hypothesis, that stress, such as suboptimal temperatures or hyposalinity, is also compensated by an increasing DPS in seaweeds.

A clear temperature-driven increase of the C:N ratio could not be determined in this study, even though it already has been reported in *L. solidungula* and other polar macroalgae (Dunton & Schell 1986, Gordillo et al. 2006, Graiff et al. 2015a). Nonetheless, at higher temperatures (10 and 15 °C), the C:N ratio was significantly increased than at lower temperatures (0 and 5 °C), showing a general negative impact of high temperatures in *L. solidungula*. Additionally, in contrast to Graiff et al. (2015a) a decrease in C:N ratio, as has been shown in *F. vesiculosus* at very high temperatures, could not be confirmed. Instead, we observed a direct correlation of temperature to the increase in C:N ratio. Atkinson & Smith (1983) showed that benthic marine algae from temperate and tropical regions had a mean C:N ratio of 20, while 10 was considered to be very low and indicative for sufficient N supply. In *L. solidungula*, the C:N ratio increased above 20 at 10 and 15 °C, indicating N limitation (Atkinson & Smith 1983, Peters et al. 2005). With the analyses of total C and total N, the strong increase in the C:N ratio could be explained by a strong decrease of total N content in the samples. The total C content

constitutes all carbohydrates, including all polysaccharides and is affected by the tissue structure (Peters et al. 2005). With changing photosynthetic activity or growth, C assimilation and C utilization can be affected (Gómez & Wiencke 1998, Gevaert et al. 2001). Nevertheless, a clear impact of temperature or salinity on the total C content could not be detected. Contrarily, the amount of total N decreased significantly with increasing temperatures, which means less N is taken up from the medium and stored as organic molecules and amino acids in the tissue (Gevaert et al. 2001). To exclude any effect of the experimental design on the reduced N uptake, sufficient N supply in the medium was ensured. In fact, the N accumulation in *L. solidungula* must therefore be intrinsically inhibited by temperature increase and at control salinity. Decreases in N concentrations might be explained, for example, by decreased nitrate reductase activity, protein synthesis and limited N storage as has been shown by Reay et al. (1999) and Gordillo et al. (2006). An increasing N uptake at lower salinities was previously detected in *Fucus serratus* and explained by an increased N metabolism at lower salinities (Gordillo et al. 2002). Mandal et al. (2015) showed a dependence of temperature and salinity on N uptake in the red alga *Kappaphycus alvarezii*. In land plants, a reduced N uptake was detected in saline soils, due to enhanced chloride concentrations in the soil (Mansour 2000). This fact can also be assigned to seaweed and seawater. Hence, an increasing N metabolism could have led to the higher N concentrations at the low salinity treatments in *L. solidungula*.

In conclusion, salinity and temperature had an additive and antagonistic impact on the Arctic seaweed *L. solidungula*, depending on the analyzed response variable. Concerning photosynthetic processes, *L. solidungula* seems to be well adapted to its Arctic habitat with natural temperature and salinity variations, but being restricted by the extreme temperature of 15 °C. *Laminaria solidungula* tolerated 0 – 10 °C and could compensate the decreasing salinities at these temperatures, while at 15 °C the osmotic stress at control salinity could not be compensated anymore. Furthermore, this study confirms that abiotic stressors can be compensated for a short period of time (e.g. Karsten 2007, Simonson et al. 2015a). Even though the absolute pigment content was not affected by the two stressors, the DPS increased to compensate rising physiological stress. Regarding mannitol, temperature increase and salinity decrease affected the concentration additively. Suboptimal temperatures resulted in lower mannitol concentrations at low salinity, while the control salinity S_A 35 resulted in higher concentrations independent from temperature. Temperatures exceeding the optimum range limited the alga's ability to compensate salinity differences. Contrary to mannitol, temperature and salinity had an antagonistic impact on total N and hence the C:N ratio, as increasing temperature

resulted in decreasing N content. Nonetheless, S_A 25 could compensate for temperature interferences.

Our results demonstrate the importance of research on physiological and biochemical responses to interactions of two or more environmental stress factors, regarding consequences of climate change. In accordance to the study of Müller et al. (2009) on polar seaweeds under ocean warming, we can confirm that also the combination of several emerging environmental stressors may result in a retreat or even extinction of some *L. solidungula* populations and a shifting into higher Arctic regions.

Acknowledgements

Open Access funding provided by Projekt DEAL. The authors are grateful to Inka Bartsch, Andreas Wagner and Claudia Daniel (Alfred Wegener Institute) for cultivating the sporophytes and for providing the facilities to conduct the experiment. This study is part the German Research Foundation for funding within the ERA-Net Cofund BiodivERsA 3 program MARFOR (ANR-16-EBI3-0005-01).

References

Andersen G, Pedersen M, Nielsen S (2013) Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima* (Laminariales, Phaeophyceae). *J Phycol* 700:689–700.

Atkinson MJ, Smith SV (1983) C:N:P ratios of benthic marine plants. *Limnol Oceanogr* 28:568–574.

Bartsch I, Paar M, Fredriksen S, Schwanitz M, Daniel C, Hop H, Wiencke C (2016) Changes in kelp forest biomass and depth distribution in Kongsfjorden, Svalbard, between 1996–1998 and 2012–2014 reflect Arctic warming. *Polar Biol* 39:2021–2036.

Bischof K (2002) Effects of solar UV-B radiation on canopy structure of *Ulva* communities from southern Spain. *J Exp Bot* 53:2411–2421.

Bischof K, Hanelt D, Aguilera J, Karsten U, Vögele B, Sawall T, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. *Mar Biol* 140:1097–1106.

Bluhm BA, Gradinger R (2008) Regional variability in food availability for Arctic marine mammals. *Ecol Appl* 18:77–96.

Celis-Plá PSM, Martínez B, Quintano E, García-Sánchez M, Pedersen A, Navarro NP, Copertino MS, Mangaiyarkarasi N, Mariath R, Figueroa FL, Korbee N (2014) Short-term ecophysiological and biochemical responses of *Cystoseira tamariscifolia* and *Ellisolandia elongata* to environmental changes. *Aquat Biol* 22:227–243.

Colombo-Pallotta MF, García-Mendoza E, Ladah LB (2006) Photosynthetic performance, light absorption, and pigment composition of *Macrocystis pyrifera* (Laminariales, Phaeophyceae) blade from different depths. *J Phycol* 42:1225–123.

Cruces E, Huovinen P, Gómez I (2012) Phlorotannin and antioxidant responses upon short-term exposure to UV radiation and elevated temperature in three south Pacific kelps. *Photochem Photobiol* 88:58–66.

Dashboard AWI (2019) Alfred Wegener Institute, Wagner C: Water Temperatures – NyÅlesund / Svalbard – N 78° 55.200 / E 11° 54.00. https://dashboard.awi.de/?dashboard=2847. Accessed 11 September 2019.

Davison IR, Greene RM, Podolak EJ (1991) Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina*. *Mar Biol* 110:449–454.

Diehl N, Michalik D, Zuccarello GC, Karsten U (2019) Stress metabolite pattern in the eulittoral red alga *Pyropia plicata* (Bangiales) in New Zealand – mycosporine-like amino acids and heterosides. *J Exp Mar Bio Ecol* 510:23–30.

Dunton KH, Schell DM (1986) Seasonal carbon budget and growth of *Laminaria solidungula* in the Alaskan High Arctic. *Mar Ecol* 31:57–66.

Eggert A, Nitschke U, West JA, Michaelik D, Karsten U (2007) Acclimation of the intertidal red alga *Bangiopsis subsimplex* (Stylonematophyceae) to salinity changes. *J Exp Mar Bio Ecol* 343:176–186.

Fernandes F, Barbosa M, Oliveira AP, Azevedo IC, Sousa-Pinto I, Valentão P, Andrade PB (2016) The pigments of kelps (Ochrophyta) as part of the flexible response to highly variable marine environments. *J Appl Phycol* 28:3689–3696.

Filbee-Dexter K, Wernberg T, Fredriksen S, Norderhaug KM, Pedersen MF (2019) Arctic kelp forests: Diversity, resilience and future. *Glob Planet Change* 172:1–14.

Fredersdorf J (2009) Interactive abiotic stress effects on Arctic marine macroalgae – Physiological responses of adult sporophytes. Doctoral Thesis. University of Bremen.

Fredersdorf J, Müller R, Becker S, Wiencke C, Bischof K (2009) Interactive effects of radiation, temperature and salinity on different life history stages of the Arctic kelp *Alaria esculenta* (Phaeophyceae). *Oecologia* 160:483–492.

Gevaert F, Davoult D, Creach A, Kling R, Janquin MA, Seuront L, Lemoine Y (2001) Carbon and nitrogen content of *Laminaria saccharina* in the eastern English Channel: biometrics and seasonal variations. *J Mar Biol Assoc U K* 81:727–734.

Gómez I, Wiencke C (1998) Seasonal changes in C, N and major organic compounds and their significance to morpho-functional processes in the endemic Antarctic brown alga *Ascoseira mirabilis*. *Polar Biol* 19:115–124.

Gordillo FJL, Aguilera J, Jiménez C (2006) The response of nutrient assimilation and biochemical composition of Arctic seaweeds to a nutrient input in summer. *J Exp Bot* 57:2661–2671.

Gordillo FJL, Carmona R, Viñegla B, Wiencke C, Jiménez C (2016) Effects of simultaneous increase in temperature and ocean acidification on biochemical composition and photosynthetic performance of common macroalgae from Kongsfjorden (Svalbard). *Polar Biol* 39:1993–200.

Gordillo FJL, Dring MJ, Savidge G (2002) Nitrate and phosphate uptake characteristics of three species of brown algae cultured at low salinity. *Mar Ecol Prog Ser* 234:111–118.

Goss R, Jakob T (2010) Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynth Res* 106:103–122.

Graiff A, Bartsch I, Ruth W, Wahl M, Karsten U (2015a) Season exerts differential effects of ocean acidification and warming on growth and carbon metabolism of the seaweed *Fucus vesiculosus* in the western Baltic Sea. *Front Mar Sci* 2:1–18.

Graiff A, Liesner D, Karsten U, Bartsch I (2015b) Temperature tolerance of western Baltic Sea *Fucus vesiculosus* – growth, photosynthesis and survival. *J Exp Mar Bio Ecol* 471:8–16.

Hanelt D, Tüg H, Bischof K, Groß C, Lippert H, Sawall T, Wiencke C (2001) Light regime in an Arctic fjord: A study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Mar Biol* 138:649–658.

Heinrich S, Valentin K, Frickenhaus S, Wiencke C (2015) Temperature and light interactively modulate gene expression in *Saccharina latissima* (Phaeophyceae). *J Phycol* 51:93–108.

Hop H, Wiencke C, Vögele B, Kovaltchouk NA (2012) Species composition, zonation, and biomass of marine benthic macroalgae in Kongsfjorden, Svalbard. *Bot Mar* 55:399–414.

Iwamoto K, Shiraiwa Y (2005) Salt-regulated mannitol metabolism in algae. *Mar Biotechnol* 7:407–415.

Jacob A, Kirst GO, Wiencke C, Lehmann H (1991) Physiological responses of the Antarctic green alga *Prasiola crispa* ssp. *antarctica* to salinity stress. *J Plant Physiol* 139:57–62.

Jacob A, Lehmann H, Kirst GO, Wiencke C (1992) Changes in the ultrastructure of *Prasiola crispa* ssp. *antarctica* under salinity stress. *Bot Acta* 105:41–46.

Ji Y, Xu Z, Zou D, Gao K (2016) Ecophysiological responses of marine macroalgae to climate change factors. *J Appl Phycol* 28:2953–2967.

Karsten U (2007) Salinity tolerance of Arctic kelps from Spitsbergen. *Phycol Res* 55:257–262.

Karsten U, Dummermuth A, Hoyer K, Wiencke C (2003) Interactive effects of ultraviolet radiation and salinity on the ecophysiology of two Arctic red algae from shallow waters. *Polar Biol* 26:249–258.

Karsten U, Thomas DN, Weykam G, Daniel C, Kirst GO (1991a) A simple and rapid method for extraction and separation of low molecular weight carbohydrates from macroalgae using high-performance liquid chromatography. *Plant Physiol Biochem* 29:373–378.

Karsten U, West JA (2000) Living in the intertidal zone – Seasonal effects on heterosides and sun-screen compounds in the red alga *Bangia atropurpurea* (Bangiales). *J Exp Mar Bio Ecol* 254:221–234.

Karsten U, Wiencke C, Kirst G (1991b) The effect of salinity changes upon the physiology of eulittoral green macroalgae from Antarctica and Southern Chile. *J Exp Bot* 42:1533–1539.

Kirst GO (1989) Salinity tolerance of eukaryotic marine algae. *Annu Rev Plant Physiol Plant Mol Biol* 40:21–53.

Koch K, Thiel M, Tellier F, Hagen W, Graeve M, Tala F, Laeseke P, Bischof K (2015) Species separation within the *Lessonia nigrescens* complex (Phaeophyceae, Laminariales) is mirrored by ecophysiological traits. *Bot Mar* 58:81–92.

Li H, Monteiro C, Heinrich S, Bartsch I, Valentin K, Harms L, Glöckner G, Corre E, Bischof K (2019) Responses of the kelp *Saccharina latissima* (Phaeophyceae) to the warming Arctic: from physiology to transcriptomics. *Physiol Plant* online.

Mandal SK, Ajay G, Monisha N, Malarvizhi J, Temkar G, Mantri VA (2015) Differential response of varying temperature and salinity regimes on nutrient uptake of drifting fragments of *Kappaphycus alvarezii*: implication on survival and growth. *J Appl Phycol* 27:1571–1581.

Mannino AM, Vaglica V, Cammarata M, Oddo E (2016) Effects of temperature on total phenolic compounds in *Cystoseira amentacea* (C. Agardh) Bory (Fucales, Phaeophyceae) from southern Mediterranean Sea. *Plant Biosyst* 150:152–160.

Mansour MFF (2000) Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol Plantarium* 43:491–500.

Maturilli M, Herber A, König-Langlo G (2013) Climatology and time series of surface meteorology in Ny-Ålesund, Svalbard. *Earth Syst Sci Data* 5:155–163.

Meredith M, Sommerkorn M, Cassotta S, Derksen S, Ekaykin A, Hollowed A, Kofinas G, Mackintosh A, Melbourne-Thomas J, Muelbert MMC, Ottersen G, Pritchard H, Schuur EAG (2019) Polar Regions. In: *IPCC Special report on the ocean and cryosphere in a changing climate*. Pörtner H-O, Roberts DC, Masson-Delmotte V, Zhai P, Tignor M, Poloczanska E, Mintenbeck K, Alegría A, Nicolai M, Okem A, Petzold J, Rama B, Weyer NM (eds) p 203–320.

Müller P, Li X-P, Niyogi KK (2001) Non-photochemical quenching. A response to excess light energy. *Plant Physiol* 125:1558–1566.

Müller R, Laepple T, Bartsch I, Wiencke C (2009) Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Bot Mar* 52:617–638.

Nitschke U, Karsten U, Eggert A (2014) Physiological performance of the red alga *Stylonema alsidii* (Stylonematophyceae) under varying salinities. *J Exp Mar Bio Ecol* 460:170–176.

Olischläger M, Bartsch I, Gutow L, Wiencke C (2012) Effects of ocean acidification on different life-cycle stages of the kelp *Laminaria hyperborea* (Phaeophyceae). *Bot Mar* 55:511–525.

Olischläger M, Iñiguez C, Koch K, Wiencke C, Gordillo FJL (2017) Increased pCO_2 and temperature reveal ecotypic differences in growth and photosynthetic performance of temperate and Arctic populations of *Saccharina latissima*. *Planta* 245:119–136.

Oliver ECJ, Donat MG, Burrows MT, Moore PJ, Smale DA, Alexander LV, Benthuysen JA, Feng M, Sen Gupta A, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell HA, Straub SC, Wernberg T (2018) Longer and more frequent marine heatwaves over the past century. *Nat Commun* 9:1–12.

Parages ML, Heinrich S, Wiencke C, Jiménez C (2013) Rapid phosphorylation of MAP kinase-like proteins in two species of Arctic kelps in response to temperature and UV radiation stress. *Environ Exp Bot* 91:30–37.

Peters KJ, Amsler CD, Amsler MO, McClintock JB, Dunbar RB, Baker BJ (2005) A comparative analysis of the nutritional and elemental composition of macroalgae from the western Antarctic Peninsula. *Phycologia* 44:453–463.

Rautenberger R, Huovinen P, Gómez I (2015) Effects of increased seawater temperature on UV tolerance of Antarctic marine macroalgae. *Mar Biol* 162:1087–1097.

Reay DS, Nedwell DB, Priddle J, Cynan Ellis-Evans J (1999) Temperature dependence of inorganic nitrogen uptake: reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. *Appl Environ Microbiol* 65:2577–2584.

Roleda MY (2016) Stress physiology and reproductive phenology of Arctic endemic kelp *Laminaria solidungula* J. Agardh. *Polar Biol* 39:1967–1977.

Russell G (1987) Spatial and environmental components of evolutionary change: interactive effects of salinity and temperature on *Fucus vesiculosus* as an example. *Helgol Meeresunters* 41:371–376.

Simonson EJ, Metaxas A, Scheibling RE (2015a) Kelp in hot water: II. Effects of warming seawater temperature on kelp quality as a food source and settlement substrate. *Mar Ecol Prog Ser* 537:105–119.

Simonson EJ, Scheibling RE, Metaxas A (2015b) Kelp in hot water: I. Warming seawater temperature induces weakening and loss of kelp tissue. *Mar Ecol Prog Ser* 61:2180–2190.

Smale DA, Wernberg T, Oliver ECJ, Thomsen M, Harvey BP, Straub, SC, Burrows MT, Alexander LV, Benthuysen JA, Donat MG, Feng M, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell HA, Sen Gupta A, Payne BL, Moore PJ (2019) Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nat Clim Change* 9:306–312.

Springer K, Lütz C, Lütz-Meindl U, Wendt A, Bischof K (2017) Hyposaline conditions affect UV susceptibility in the Arctic kelp *Alaria esculenta* (Phaeophyceae). *Phycologia* 56:675–685.

Sundfjord A, Albretsen J, Kasajima Y, Kohler J, Nuth C, Skarðhamar J, Cottier F, Nilsen F, Asplin L, Gerland S, Torsvik T (2017) Effects of glacier runoff and wind on surface layer dynamics and Atlantic water exchange in Kongsfjorden, Svalbard; a model study. *Estuar Coast Shelf Sci* 187:260–272.

Svendsen H, Beszczynska-Møller A, Hagen JO, Levauconnier B, Vigdis T, Gerland S, Børre Ørbæk J, Bischof K, Papucci C, Zajaczkowski M, Azzolini R, Bruland O, Wiencke C, Winther JG, Dallmann W (2002) The physical environment of Kongsfjorden-Krossfjorden, an Arctic fjord system in Svalbard. *Polar Res* 21:133–166.

Thomas DN, Collins JC, Russell G (1988) Interactive effects of temperature and salinity upon net photosynthesis of *Cladophora glomerata* (L.) Kütz. and *C. rupestris* (L.) Kütz. *Bot Mar* 31:73–77.

tom Dieck (Bartsch) I (1992) North Pacific and North Atlantic digitate *Laminaria* species (Phaeophyta): Hybridization experiments and temperature responses. *Phycologia* 31:147–163.

tom Dieck I (1989) Vergleichende Untersuchungen zur Ökophysiologie und Kreuzbarkeit innerhalb der digitaten Sektion der Gattung *Laminaria* Lamouroux (Phaeophyceae). Doctoral Thesis. University of Hamburg.

Verardo DJ, Froelich PN, McIntyre A (1990) Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 Analyzer. *Deep Res* 37:157–165.

Walczowski W, Piechura J, Goszczko I, Wieczorek P (2012) Changes in Atlantic water properties: An important factor in the European Arctic marine climate. *ICES J Mar Sci* 69:864–869.

Wiencke C, Clayton MN, Gómez I, Iken K, Lüder UH, Amsler CD, Karsten U, Hanelt D, Bischof K, Dunton K (2007) Life strategy, ecophysiology and ecology of seaweeds in polar waters. *Life Extrem Environ* 213–244.

Zacher K, Rautenberger R, Hanelt D, Wulff A, Wiencke C (2009) The abiotic environment of polar marine benthic algae. *Bot Mar* 52:483–490.

Supplement

Table S6.1 Results of the generalized linear model (GLM) for effects of temperature and salinity on pigments (chlorophyll *a*, fucoxanthin, chlorophyll *c*2, β-carotene) and the pool size of the xanthophylls (VAZ = violaxanthin, antheraxanthin, zeaxanthin) (μg g^{-1} DW) of *Laminaria solidungula*. Statistically significant values are indicated by asterisks ($p < 0.05$).

Variable	Factor	*df*	*p* value
Chlorophyll *a*[a]	Temperature	3	0.002405*
	Salinity	1	0.027089*
	Temperature × Salinity	3	0.215918
Fucoxanthin[a]	Temperature	3	0.18823
	Salinity	1	0.04071*
	Temperature × Salinity	3	0.25380
Chlorophyll *c*2	Temperature	3	0.05063
	Salinity	1	0.21829
	Temperature × Salinity	3	0.94155
β-Carotene[a]	Temperature	3	0.00172*
	Salinity	1	0.49941
	Temperature × Salinity	3	0.73629
VAZ[b]	Temperature	3	1.804e-05*
	Salinity	1	0.6962
	Temperature × Salinity	3	9.205e-05*

[a] reciprocal [b] $\log_{10}$ transformation

7. Synoptic Discussion

7.1. *Saccharina latissima* along the latitudinal gradient – Global patterns vs. microclimates

Broadly distributed species are often treated as single homogenous physiological units (Reed et al. 2011). However, this study clearly revealed that *Saccharina latissima* must not be regarded as such. Despite the fact that the studied species' genetic diversity across latitudes in Europe was found to be low (Guzinski et al. 2020), it exhibits marked habitat-specific differences in morphological, physiological and biochemical profiles that vary in function of local variation in temperature, irradiance or salinity. The biogeography of kelp is mainly controlled by temperature (Lüning 1984, Adey & Steneck 2001), but thermal tolerance ranges and optima vary between species (Bartsch et al. 2008, Müller et al. 2009). Local adaptation and phenotypic plasticity eventually result in different thermal tolerances between populations and which may lead to the influence of ocean warming being underestimated (Bartsch et al. 2013, King et al. 2018a, 2019, Bennett et al. 2019, Filbee-Dexter et al. 2020). In a study on temperature tolerance along a latitudinal gradient, Bennett et al. (2015) demonstrated that the rear edge and central populations of the Australian brown macroalga *Scytothalia* sp. exhibit equal sensitivity to warming. To date, most laboratory studies on temperature stress have not considered variation in local temperature and thus do not reflect realistic ecological conditions (e.g. Bolton & Lüning 1982, Pereira et al. 2015, King et al. 2019, Liesner et al. 2020a, Martins et al. 2020). For future prognoses of changes in marine forests, however, more comprehensive approaches will be important.

For that reason, in **publication I** local marine heatwave (MHW) events were simulated with field sporophytes of *S. latissima* from five locations along the European Atlantic coast. No homogenous responses to the temperature increases realistically expected during summer were detected. Thus, *S. latissima* is not affected equally by MHWs across all latitudes. Habitat-specific differences in physiological and biochemical responses were found between the populations from Spitsbergen, Bodø, Bergen, Helgoland and Locmariaquer. Harmful impacts were exclusively detected in the rear-edge populations and only when the treatments surpassed 20 °C. The expanding-edge populations were overall unaffected by MHWs and did not benefit from increasing temperatures. Hence, **publication I** contradicts the assumption of homogenous thermal susceptibility of different *S. latissima* populations (Reed et al. 2011). The findings, however, also provide evidence that models that assume a uniform climatic envelope for *S. latissima* do not generally underestimate survival regarding MHW events in cold-temperate and Arctic regions as has been proposed, e.g. by Kelly et al. (2012) or Filbee-Dexter et al.

(2020). In contrast to earlier reports on the upper thermal survival limits of the species (Fortes & Lüning 1980, Bolton & Lüning 1982), samples from Helgoland (German Bight) and Locmaria-
quer (Brittany) survived in temperatures up to 25 °C for more than a week (**publication I**), most probably due to the stepwise temperature increase, mimicking MHWs in nature (Terblanche et al. 2007, Hobday et al. 2016). Additionally, the stress responses exhibited different plasticity to relative temperature increase: Strong site-specific biochemical and physiological phenotypes were detected, however, these were not explicitly attributable to geographical origins (**hypothesis I**). For instance, independent of the applied temperature increase, samples from Spitsbergen exhibited markedly higher growth activity in summer than all other populations (**publication I**). This shows that within-species variation is indeed overlooked in *S. latissima* (Bennett et al. 2019, Filbee-Dexter et al. 2020).
The findings of intraspecific variation are strongly supported by the results of **publication IV**, for which the biochemical and morphological variability of *S. latissima* across its entire distribution range in Europe were investigated. Therefore, links between biochemical composition and morphology of adult field sporophytes, and the respective local abiotic conditions along latitudinal and salinity gradients, were analyzed. Strong dependency of morphological and biochemical traits on local abiotic conditions could be determined, confirming that different phenotypes indeed provide information on their respective environmental conditions (Martins et al. 2014, Monteiro et al. 2020). Thus, the geographical origin is reflected in the specific phenotypes to a certain extent, although *S. latissima* did not exhibit habitat-specific signatures in their biochemical profile (**hypothesis I**).

Selective effects can generate genetic diversity along latitudinal gradients (Parmesan 2006). For instance, *Laminaria digitata*, which is also a broadly distributed cold-temperate kelp species, revealed a unique gene pool at low latitudes in the North East Atlantic, as a direct result of the expansion of the Eurasian ice sheet during the last glaciation period (Neiva et al. 2020). Likewise, glacial vicariance is reported to have led to phylogeographic diversification in *S. latissima*, resulting in two large clusters in the North East Atlantic (Neiva et al. 2018). The northern phylogenetic group includes Spitsbergen and southern Norway, Iceland, Greenland and Russia, and the southern one, Brittany and the Iberian Coast (Neiva et al. 2018). Further genetic analyzes using specific microsatellites revealed distinct differentiation of *S. latissima* populations within Europe even across small geographical distances but no significant latitudinal variation (Guzinski et al. 2016, 2020).

For kelps, first species and population assignments are enabled by barcoding the mitochondrial cytochrome-*c*-oxidase I gene (COI) (Neiva et al. 2018). For **publication IV**, I tested whether COI-haplotypes were sufficient to detect distinct genetic clusters in *S. latissima* according to their geographical origin and phenotypical profiles, and confirmed variability of the COI gene in the warm-edge populations. Populations from Helgoland, southern Brittany and the Iberian Coast revealed slight differences in their COI barcodes compared to other locations. These results are in accordance with the northern and southern phylogenetic clusters in Neiva et al. (2018). Significant physiological, biochemical and genetic differences between brackish and marine populations were reported by Nielsen et al. (2014) and (2016a,b). Additionally, major impact of the salinity gradient in the Baltic Sea on morphological and biochemical phenotypes of *S. latissima* were determined, however, the populations were indistinguishable from the fully marine populations using mitochondrial COI markers (**publication IV**). Thus, *S. latissima* from the Baltic Sea does not constitute its own phylogenetic cluster, separate from other North East Atlantic populations (northern cluster). Genetic variability of brackish compared to marine populations was only distinguishable using microsatellite markers (Nielsen et al. 2016b). In summary, COI-haplotypes that represent 'genetic phenotypes' separate into northern and southern phylogenetic clusters, but distinct geographical origin is not discernable (**hypothesis I**).

7.1.1. Variability over time – Inter-annual vs. seasonal variation and the significance of marine heatwaves

Publication I demonstrated that marine heatwaves (MHWs) in summer have different effects on *Saccharina latissima* populations across latitudes. Concomitantly, it was validated that different abiotic conditions have a strong impact on physiological and biochemical traits of the species in general (**publication I & IV**). As a so-called 'season responder', *S. latissima* adjusts towards favorable temperature and light conditions throughout the year (Dunton 1985, Wiencke et al. 2009) and therefore exhibits seasonal patterns in physiology and biochemical composition (e.g. Sjøtun 1993, Bischof et al. 2002, Andersen et al. 2011, Schiener et al. 2015). However, some environmental factors, such as sea surface temperature (SST), do not only vary between seasons but also between years. It was suggested that warmer years increase local kelp mortality since severe losses of kelp populations were observed after extremely high SSTs in the North Atlantic (Andersen et al. 2013, Filbee-Dexter et al. 2020).

Therefore, the impact of seasonal and inter-annual SST variation on the sensitivity of *S. latissima* to marine heatwaves (MHWs) in summer were investigated in **publication II**. The findings confirmed that the upper thermal tolerance of *S. latissima* differs between seasons and years and indeed is dependent on SST variation. Field sporophytes were more sensitive to simulated MHWs at the end of summer and during the extremely warm year. We also showed that the seasonal variation (June vs. August 2018) was greater than the inter-annual effect (2018 vs. 2019), demonstrating that temperature increase has a stronger impact on *S. latissima* after longer periods of high SST and more frequent MHWs. Accordingly, **hypothesis II** was confirmed: Seasonal thermal history determines the tolerance of *S. latissima* to MHWs. Consequentially, reductions and extinctions of kelp populations will be more likely to occur in warmer years when upper survival limits are surpassed, as proposed by Andersen et al. (2013) and Filbee-Dexter et al. (2020). However, diverse unbalanced multitrophic interactions that result from extensive MHWs can also lead to the collapse of kelp ecosystems

Seasonal variation in the upper survival temperature of *S. latissima* field sporophytes from Helgoland as previously described by Lüning (1984) but no inter-annual variation was observed. Contrary to the findings in **publication II**, lower tolerance was detected in early spring compared to in summer. Additionally, the survival limits between 18 – 20 °C were much lower compared to the observations in **publication I and II**, possibly due to seasonal SST fluctuations. The SST around Helgoland can vary from −1 °C to 21 °C throughout the year (Boersma et al. 2016), and the cold season has a major influence on the thermal performance of kelps. For example, positive effects of low temperatures during the gametogenesis and recruitment phases on the thermal plasticity were recently reported for juvenile *Laminaria digitata* sporophytes (Liesner et al. 2020b). Prior studies also demonstrated that temperature history affects enzymatic activity and, therefore, is an important factor in the regulation of the photosynthetic metabolism of *S. latissima* (Davison 1987, Davison & Davison 1987), e.g. higher photosynthetic capacity was measured in *S. latissima* grown at 0 °C, compared to those grown at 20 °C (Davison 1987).

Thus, cold winter and spring temperatures have the potential to counteract high temperatures later in the year and, accordingly, declines in marine forests will mainly occur in regions that experience higher temperatures throughout the entire year. Concomitantly, ocean warming over winter (IPCC 2019) will impair stress resilience in kelps, though MHWs during the cold seasons may not lead to an immediate loss of populations. By affecting the thermal plasticity of kelp, not only rear-edge populations but also central populations may thus be endan-

gered. Accordingly, aside from summer thermoclines which have a direct impact on kelp populations, winter thermoclines with their impact on stress resilience are of major importance to kelp distribution (Müller et al. 2009, Assis et al. 2018). Future modeling approaches should therefore account for the entire environmental thermal history.

7.1.2. Local adaptation or phenotypic plasticity in *Saccharina latissima*

Saccharina latissima exhibits high plasticity in morphology, physiology and biochemistry and can thus acclimate to a broad range of abiotic conditions (reviewed by Bartsch et al. 2008). Genetic differentiation between several populations within the North East Atlantic phylogenetic group has been identified in several studies (Guzinski et al. 2016, 2020, Nielsen et al. 2016b, Luttikhuizen et al. 2018, Neiva et al. 2018). These genetic differences provide prerequisites for the emergence of ecotypes (King et al. 2018a, Guzinski et al. 2020), which can develop after long-term exposure to selective environmental pressures, such as temperature or salinity (Nicotra et al. 2010). Thermal ecotypes were recently confirmed, although only subtle differences were found for the likewise broadly distributed kelp *Laminaria digitata* (King et al. 2019, Liesner et al. 2020a, Martins et al. 2020). Several studies– based on different physiological and biochemical traits – have postulated ecotypic differentiation regarding temperature, salinity and light in *S. latissima* from the North East (Müller et al. 2008, Olischläger et al. 2014, 2017) and North West Atlantic (Gerard et al. 1987, Gerard 1988, 1990, Gerard & Du Bois 1988). *Saccharina latissima* was proposed to separate into Arctic and temperate ecotypes based on a variety of approaches regarding set-ups and target parameters (Lüning 1975, Gerard & Du Bois 1988, Müller et al. 2008, Olischläger et al. 2014, 2017). Yet, ecotypic differentiation did not persist in the next generation after the hybridization of two populations (Lüning 1975, Müller et al. 2008). Bolton & Lüning (1982) suggested high phenotypic plasticity in *S. latissima* rather than thermal ecotypes with respect to growth and survival of gametophytes and sporophytes from several locations in Europe. However, it is known that locally-adapted ecotypes can also exhibit high phenotypic plasticity (de Jong 2005).

Many studies that have proposed thermal ecotypes based their conclusion on studies with field sporophytes (e.g. Lüning 1975, Gerard et al. 1987, Gerard & Du Bois 1988). In this study, physiological and biochemical traits of field sporophytes were shown to exhibit different plasticity to temperatures (**publication I & II**) and habitat-specific variability independent of latitudinal distribution (**publication I & IV**). These findings prove that studying field samples

impedes the direct comparison of different populations or experiments. Common garden experiments, like those conducted by Liesner et al. (2020a) or Martins et al. (2020) on *L. digitata*, are needed to definitely clarify the existence of local adaptations or ecotypes in *S. latissima*. Therefore, Li et al. (2020a) and Monteiro et al. (2021) conducted common garden experiments by applying the same temperatures and salinities on *S. latissima* from Roscoff (Brittany) and the Arctic (Spitsbergen). Sporophytes from both locations were grown under exactly the same laboratory conditions. The studies revealed supporting evidence for the existence of ecotypes in *S. latissima*. The sporophytes exhibited distinct variability in morphology and an overall stronger response was found at low temperatures (0 °C) in samples from Roscoff, while Arctic samples responded more strongly at higher temperatures (15 °C). However, Arctic sporophytes did not grow better at low temperatures and Roscoff sporophytes at higher temperatures, as expected for thermal ecotypes, thus, definite ecotypic differentiation could not be confirmed (Monteiro 2020). Thus, the question whether *S. latissima* exhibits distinct thermal ecotypes still remains unanswered and further research is needed. Luttikhuizen et al. (2018) hypothesized that *S. latissima* in Europe has not yet reached an equilibrium of fixation in genetic differentiation. This is expected to eventually occur if population structures are left undisturbed, potentially resulting in the evolution of ecotypes or even further separation within the species complex (Bolton 2010, Luttikhuizen et al. 2018). Reaching an equilibrium level, however, might be precluded by rapid alterations in habitats due to climate change.

In conclusion, **publication I, II and IV** demonstrated that the hypothesized local adaptations have no ecological implications on *S. latissima* sporophytes with regards to increasing temperatures in summer. Instead, the annual variability of ambient temperatures affects the thermal susceptibility of sporophytes more severely. Nevertheless, habitat-specific differentiation was observed, proving that intraspecific variation is indeed overlooked (Bennett et al. 2019, Filbee-Dexter et al. 2020), even without clearly distinguishable ecotypes.

7.2. Implications for marine forests along the European coastline

Marine forests are of high ecological and economical relevance given their extensive ecosystem services. Their geographical distribution, particularly at the trailing edge, is majorly controlled by high temperatures, as is expected from its cold-water adaptation. Though nutrient limitation or competition from other seaweeds also affect their distribution by influencing rear-edge populations, whereas the expanding edge distribution is more defined by light limitation (Lüning 1984, Adey & Steneck 2001, Steneck et al. 2002). Yet, climate change has far-reaching impacts on marine forest ecosystems (IPCC 2019, Smale 2020), threatening the diversity and resulting in shifts into novel ecosystem states (Frölicher et al. 2018, Harris et al. 2018, King et al. 2018b, Smale et al. 2019).

Saccharina latissima is one of the dominating kelp species along the European Atlantic Coast (Araújo et al. 2016). Large northward shifts are predicted for the species due to the ongoing borealization of the Arctic (Müller et al. 2009, Assis et al. 2018). **Publication I** demonstrated that short-term temperature increases in summer alone will not lead to an expansion of leading-edge populations, as specimens from Spitsbergen did not benefit from heatwave-like events in my experiments. In contrast, at the expanding edge, enhanced temperatures were ascertained to primarily contribute to the decline or even total loss of rear-edge kelp populations (Pinho et al. 2016, Wernberg et al. 2016, Assis et al. 2017, Burdett et al. 2019, Filbee-Dexter et al. 2020). Accordingly, southern populations of *S. latissima* from Brittany and Iberia are expected to disappear towards the end of this century (Müller et al. 2009, Assis et al. 2018). Sporophytes of *S. latissima* were reported to survive in temperatures up to 23 °C for a week, with mortality increasing at > 20 °C (Fortes & Lüning 1980, Bolton & Lüning 1982). Correspondingly, the modeling studies by Müller et al. (2009) set southern summer thermoclines at 19 – 21 °C and Assis et al. (2018) set physiological upper temperature tolerance limits of *S. latissima* at 20.9 °C in summer and also accounted for other factors, such as sea ice, salinity and upwelling. The findings in **publication I** revealed that a gradual increase in temperature allows rear-edge populations of *S. latissima* to endure temperatures up to even 25 °C for about two weeks, with increasing mortality only at temperatures > 23 °C. These findings, however, just relate to sporophytes. Gametophytes of Laminariales generally exhibit 3 – 4 °C higher thermal limits than sporophytes (tom Dieck 1993). Hence, even though Assis and colleagues also accounted for discrepancies in the physiological performance of different life-cycle stages, the thermal tolerance of southern populations was potentially underestimated

in both modeling approaches (Müller et al. 2009, Assis et al. 2018). Nevertheless, in **publication II**, we demonstrated that yearly seasonal temperature history does alter thermal susceptibility of *S. latissima* to marine heatwaves (MHWs). Longer periods of high temperatures or more MHW days led to lower thermal tolerances of field sporophytes from Helgoland. Consequentially, prospective increased frequency and duration of MHWs (Oliver et al. 2018) will impair kelp populations, despite the determined higher thermal limit (**publication I**). Additionally, *in situ* variability in morphology and biochemical profiles of *S. latissima* (**publication IV**) generate different biochemical prerequisites to withstand temperature fluctuations. Yet, it should be noted that experiments in this study solely simulated short-term temperature increases in summer with sufficient nutrient supply (½ PES) (**publication I & II**). In the future, long-term experiments applying successive increases in temperature under ambient nutrient conditions will therefore be indispensable for robust predictions of the impact of ocean warming on the future distribution patterns of *S. latissima* populations. Further integration of empirical and modeling approaches will ultimately allow for better forecasts of the environmental impacts on marine forests (Franco et al. 2018).

Still, the loss of marine forests, reduced extension and range contractions in Europe have already been reported in reams of studies (Bekkby & Moy 2011, Tuya et al. 2012, Raybaud et al. 2013, Voerman et al. 2013, Krumhansl et al. 2016, Filbee-Dexter 2020, Saha et al. 2020). This is certainly because temperature increases affect multifarious aspects of kelp ecosystems (Harley et al. 2006, 2012). Reproduction of *Laminaria digitata*, for instance, is inhibited at 20 °C while fertility declines even between 18 – 19 °C (Bartsch et al. 2013). However, low temperatures during early development are also known to constitute a large component of the thermal plasticity of kelp sporophytes (Liesner et al. 2020b) and rapid temperature increases also occur in winter (IPCC 2019). Furthermore, unbalanced multitrophic interactions of keystone predators, such as cod, crabs, sea otters and sea stars, that arise from sudden temperature increases, can potentially contribute to kelp ecosystem collapses and regime shifts to 'sea urchins barrens' (Christie et al. 2019b, Rogers-Bennett & Catton 2019, McPherson et al. 2021). Additionally, differences in thermal tolerances of macroalgal species can lead to outcompeting of less-tolerant species (Krause-Jensen & Duarte 2014, Armitage et al. 2017, Straub et al. 2019).

It was proposed that in addition to temperature, multiple other abiotic factors contribute to the decline of kelp communities and that interactions between these factors affect resistance and resilience, e.g. between grazing and competition (Schiel et al. 2004, Wernberg et al. 2010,

Andersen et al. 2013). For instance, shifts from *S. latissima* dominated communities to algal turf communities have been observed in Europe, driven by increasing temperatures and eutrophication (Christie et al. 2009, 2019a, Andersen et al. 2011, Moy & Christie 2012). **Publication III and V** (see chapter 7.3) confirmed that the interactive effects between different abiotic factors should not be underestimated in future distribution models. Thus, simultaneous changes in environmental – abiotic and biotic – variables indicate an uncertain future for marine forests. Certainly, a fundamental understanding of the intra- and interspecific response mechanisms of foundation species to variation in different drivers is essential.

7.3. The future of Arctic marine forests under the impact of multiple drivers

Local variations in abiotic factors drive the morphology and the biochemical profile of adult field sporophytes. **Publication IV** highlighted how strongly *Saccharina latissima* is affected by the respective physico-chemical settings in its habitat. Arctic kelp species are usually exposed to very low temperatures and extreme seasonal variation in light climate and sea ice coverage. However, due to climate change, the Arctic is one of the most rapidly changing regions in the world (Maturilli et al. 2013, Bischof et al. 2019, Meredith et al. 2019). Apart from temperature increases, the marine ecosystem is now undergoing dramatic declines in sea ice and increases in freshwater input and terrestrial-run-offs (Sundfjord et al. 2017), resulting in changing salinity, nutrient input and underwater light regimes (Hanelt et al. 2001, Filbee-Dexter et al. 2019). Only little is known on the effects of interacting factors on Arctic seaweeds (Fredersdorf et al. 2009, Springer et al. 2017, Li et al. 2020a), despite the fact that their interplay might have synergistic or antagonistic impacts on the resilience and thermal susceptibility of kelps.

To shed light on the interactive effects of temperature increases and hyposaline or nutrient-enriched conditions in Arctic seaweeds, different two-factorial stress experiments were conducted on field sporophytes of *S. latissima* (**publication III**: temperature × salinity and temperature × nutrients) and young sporophytes of *Laminaria solidungula* (**publication V**: temperature × salinity). Evaluating the impact of the abiotic factors individually, *S. latissima* exhibited slightly enhanced growth activity and vitality under realistic Arctic temperature increase (**publication III**). These findings are in accordance with their optimum temperature range, which is between 10 and 15 °C (Bolton & Lüning 1982) and previous reports on Arctic *S. latissima* (Iñiguez et al. 2016, Li et al. 2020a). In contrast to *S. latissima*, *L. solidungula* is

very well adapted to low temperatures (tom Dieck [Bartsch] 1992, Bartsch et al. 2008), and thus, samples revealed clear indications of heat stress when their optimum range of 5 – 10 °C of was exceeded (tom Dieck [Bartsch] 1992) (**publication V**). Variation in salinity can lead to osmotic stress in seaweeds, affecting both their physiological and biochemical status (Gerard et al. 1987, Lüning 1990, Gordillo et al. 2002, Karsten 2007). However, I can clearly rule out that hyposaline conditions alone, as they frequently occur in Arctic fjords, considerably affect the two kelp species (**publication III & V**).

However, Nielsen et al. (2014) proposed that low salinity in combination with high summer temperatures might contribute to changes in the physiological status of *S. latissima* in the Baltic Sea. A pronounced temperature effect, low impact of salinity and minor interactive effects were also detected in the early life stages of *S. latissima* (Li et al. 2020a, Monteiro et al. 2021). Contrary to these studies, no interaction between temperature and salinity in Arctic field sporophytes was identified (**publication III**). **Hypothesis III** must therefore be partially rejected: Hyposalinity does not affect the thermal susceptibility of Arctic *S. latissima*, at least not in the tested – realistic – range. However, it is known that laboratory cultures and young sporophytes, as studied by Li et al. (2020a) and Monteiro et al. (2021), are generally more sensitive to changes in the environment than field sporophytes (Hanelt et al. 2001, Heinrich et al. 2015, 2016). In accordance with **publication III**, field sporophytes of Arctic *Alaria esculenta*, another broadly distributed boreal-temperate kelp species, also did not show significant temperature × salinity interaction effects (Fredersdorf et al. 2009). Furthermore, Li et al. (2020a) and Monteiro et al. (2021) revealed that interactive effects were less evident in the physiology than in the transcriptomics of *S. latissima*. In **publication III**, only investigated the main physiological and biochemical traits were analyzed and might be another reason why no temperature × salinity interactions were found. Even so, **publication V** revealed strong physiological and biochemical responses to interactive temperature and salinity treatments in young laboratory-grown *L. solidungula*, thus partially confirming **hypothesis III**: Hyposalinity affects the thermal susceptibility of *L. solidungula*. Complex interactions of temperature and salinity led to both synergistic and antagonistic effects, alternating in the different response parameters (**publication V**).

No distinct responses to temperature increases under enhanced nutrient conditions in Arctic *S. latissima* could be identified (**publication III**). None of the examined parameters were affected by artificial nutrient enrichment alone, in accordance with the study of Gordillo et al. (2006). They reported that the Arctic kelp species are not nitrogen-limited, although primary production is often limited by low nutrient availability in summer (Filbee-Dexter et al. 2019).

Even in combination with increased temperatures, enriched nutrient conditions did not significantly improve the status of *S. latissima*, though appear to slightly support its growth and vitality. Thus, the expected enhanced nutrient availability during Arctic summers will not strongly affect the thermal susceptibility of Arctic *S. latissima* (**hypothesis III**).

Over the past decades, Arctic coastal ecosystems have already experienced major changes in marine vegetation, i.e. the expansion of geographical distribution, increase in abundance, productivity or species richness (Krause-Jensen et al. 2020). In Kongsfjorden (Spitsbergen), massive changes in the benthic community were reported between 1996/1998 and 2012/2013 (Hop et al. 2012, Bartsch et al. 2016). *Saccharina latissima*, amongst the dominating species, experienced slight increases in biomass and reductions in depth extension and the less abundant *L. solidungula* also showed decreased depth distribution over time. Further changes in geographical distribution and abundance are projected with the ongoing changes in the environment (Müller et al. 2009, Krause-Jensen & Duarte 2014, Assis et al. 2018). Some studies estimated that temperature increase could even more than double the kelp production in some Arctic regions within the next twenty to thirty years (Filbee-Dexter et al. 2019). However, diversity may be lost if the temperate-adapted algal communities outcompete more sensitive species (Krause-Jensen & Duarte 2014). Species with wide distributional ranges and thus larger temperature tolerances, such as *S. latissima*, thus may successfully spread, but at the cost of ecological diversity (Wiencke et al. 1994, Kelly et al. 2012, Mineur et al. 2015, Sunday et al. 2015).

Different model studies predict the ongoing borealization of southern Arctic habitats and hence further poleward expansion of *S. latissima* and *L. solidungula*, but concomitantly, the loss of *L. solidungula* populations at their southern range limit (Müller et al. 2009, Assis et al. 2018). From my work, however, I can neglect that expansion of *S. latissima* could be solely based on short-term temperature increases, like during marine heatwaves (MHWs) in summer (**publication I**). Considering interactive environmental factors, Arctic *S. latissima* (**publication III**) and *L. solidungula* (**publication V**) both generally tolerate the changes predicted for the Arctic, although responses accrete with increasing pressure. In this context, **publication III and V** revealed that interactions can have complex beneficial and impairing effects on Arctic kelp species. In accordance with a study by Wiencke et al. (1994) on the temperature tolerance of seaweeds, I could show that the Arctic endemic *L. solidungula* was more sensitive to environmental change than the broadly distributed *S. latissima* (**publication III & V**). The findings confirm that *S. latissima* will likely increase in abundance under realistic climate

change scenarios in the Arctic and has the potential to outcompete more sensitive species, such as *L. solidungula*, resulting in subsequent changes in biodiversity.

Still, when extrapolating my findings on Arctic marine forests, it has to be considered that the responses of *L. solidungula* (**publication V**) were based on young laboratory cultures, while the sporophytes studied of *S. latissima* were fully-grown and field-collected (**publication III**). Furthermore, the traits investigated in this study focused on the main physiological and biochemical response mechanisms of the species. Other consequences, such as decreased spore settlement and gametophyte growth as was shown at elevated temperatures and low salinity for *S. latissima* (Lind & Konar 2017) could still occur.

Additionally, temperature, salinity and nutrients are not the only drivers changing in Arctic ecosystems. For instance, the light regime is also continuously changing and might further impact Arctic kelps on multiple levels (Wiencke et al. 2006, Spurkland & Iken 2012, Filbee-Dexter et al. 2019). Scheschonk et al. (2019) compared the ability of *S. latissima* and *L. solidungula* to resist polar night conditions at increasing temperatures. Pronounced variability in physiological and biochemical traits was observed, primarily in the long-term carbon storage compound laminarin, indicating the adaptation of *L. solidungula* to long periods of darkness and low irradiance. However, downregulating transcriptomes to reduce energy consumption are potential adjustments of Arctic *S. latissima* populations to darkness (Li et al. 2020b). The ongoing decline in sea ice coverage enables earlier light transmission, shifting seasonal timing (Bischof et al. 2019). This might allow 'season responders', such as *S. latissima*, to grow earlier in the year, resulting in direct competition with the 'season anticipator' *L. solidungula*. Projecting the current state of knowledge from both my studies (**publication III & V**) and the work of others, *S. latissima* will likely outcompete *L. solidungula*, resulting in a decline in diversity in Arctic marine forests and potentially even a change in Arctic ecosystem functions. To summarize, the findings of my thesis highlight the importance of further investigation of the impact of abiotic interactions on Arctic marine forests.

7.4. Conclusion and future perspectives

Reflecting on the underlying hypotheses, the following can be concluded:

Hypothesis I: **The geographical origin of *Saccharina latissima* is reflected in different phenotypes, and sporophytes exhibit habitat-specific signatures.**

The hypothesis can be partially confirmed: The geographical origin of *S. latissima* is, to a certain extent, reflected in different morphological, biochemical and genetic phenotypes, although sporophytes do not exhibit habitat-specific signatures.

Hypothesis II: **The seasonal thermal history determines the tolerance of *Saccharina latissima* to marine heatwaves.**

This hypothesis can be confirmed: Seasonal thermal history changes the thermal susceptibility of *S. latissima* to marine heatwaves.

Hypothesis III: **Hyposalinity and nutrient enrichment affect the thermal susceptibility of kelps from the Arctic.**

This hypothesis must be partially rejected but can also be partially confirmed: Realistic hyposalinity does not affect the thermal susceptibility of Arctic *Saccharina latissima*, but it affects the thermal susceptibility of the endemic *Laminaria solidungula*. Enhanced nutrient availability during Arctic summers does not particularly affect the thermal susceptibility of Arctic *S. latissima*.

In some marine barren regions, the natural recovery from previous kelp decline was observed decades ago (Pearse & Hines 1979). However, ongoing climate change exacerbates the status of kelps globally. Innovative and integrative management is needed to counteract the collapse of marine forests (Bonebrake et al. 2018, Jueterbock et al. 2021).

Coastal conservation strategies include, for example, the translocation of kelp into deforested areas (Andersen et al. 2011) or sophisticated management of local pressures (Strain et al. 2015). Still, conservation approaches, including 'assisted evolution' must be managed with extreme caution, otherwise they risk provoking the elicitation of damaging processes (Filbee-Dexter & Smajdor 2019). Changes in the seaweed diversity may have an effect on ecosystem functions, even if the ecological niche of foundation species is filled by more robust species (Mineur et al. 2015). Therefore, a better understanding of genetic diversity, as well as the physiological and biochemical mechanisms in various kelp species, is of major importance.

The aim of this study was to provide knowledge on the acclimation patterns of foundation species to temperature increases and interacting abiotic drivers along large geographical and environmental gradients. The findings clearly demonstrated that the broadly distributed kelp *S. latissima* cannot be regarded as a single homogenous physiological unit and within-species variation must not be underestimated. Habitat-specific differences in morphology, physiology and biochemical composition were found across the entire distribution range of *S. latissima* in Europe (**publication I & IV**). **Publication I** also revealed that expected short-term temperature increases in summer do not equally affect *S. latissima* populations across latitudes. The hypothesized thermal ecotypes are negligible with regards to marine summer heatwaves, and instead, temperature history early in the year was confirmed to alter the thermal susceptibility of *S. latissima* (**publication I & II**). Ocean warming in the winter and early spring season might particularly affect stress resilience and hence contribute to the reduction of kelp that has already been observed and projected in Europe. Further studies that focus especially on environmental changes in winter are needed here for a better understanding of how kelps will respond to climate change. Moreover, previous studies have reported different temperature responses between life-stages (tom Dieck 1993), gametophyte sexes (Monteiro et al. 2019) or thallus age (Lüning 1984), all of which will need to be investigated across latitudes.

Interactions between different environmental factors are known to be very complex. The nature of interactive effects can vary between species, life stages and response variables but also across latitudes (e.g. Fredersdorf et al. 2009, Heinrich et al. 2015, Martins et al. 2017, Christie et al. 2019b). **Publication III and V** demonstrated the importance of research on the

responses of kelps to interacting factors, especially regarding realistic ecological aspects and against the background of climate change. *Saccharina latissima* and the Arctic endemic *Laminaria solidungula* exhibited different sensitivity to drivers related to environmental change (**publication III & V**). The fact that even within the same species, the interaction of abiotic factors can generate both synergistic and antagonistic effects (**publication V**) highlights its need to be elaborated on in future approaches. Further knowledge on the interaction of drivers in different seaweeds and its integration into conservation approaches will be key for maintaining marine forests in the North East Atlantic, if not across the entire globe.

8. Reference List for the Synoptic Chapters

Acker JG, Leptoukh G (2007) Giovanni Satellite: Online analysis enhances use of NASA Earth Science Data. *Eos, Trans Am Geophys Union* 88:14–17.

Adey WH, Steneck RS (2001) Thermogeography over time creates biogeographic regions: A temperature/space/time-integrated model and an abundance-weighted test for benthic marine algae. *J Phycol* 37:677–698.

Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. *Mar Biol* 140:1087–1095.

Alfred Wegener Institute (2019) Water Temperatures – NyÅlesund/Svalbard – N 78°55.200 / E 11°54.00. https://dashboard.awi.de/?dashboard=2847.

Amsler CD (2008) Algal chemical ecology. Amsler CD (ed). Springer-Verlag, Berlin Heidelberg.

Andersen G, Pedersen M, Nielsen S (2013) Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima* (Laminariales, Phaeophyceae). *J Phycol* 49:689–700.

Andersen GS, Steen H, Christie H, Fredriksen S, Moy FE (2011) Seasonal patterns of sporophyte growth, fertility, fouling, and mortality of *Saccharina latissima* in Skagerrak, Norway: Implications for forest recovery. *J Mar Biol* 2011:690375.

Araújo RM, Assis J, Aguillar R, Airoldi L, Bárbara I, Bartsch I, Bekkby T, Christie H, Davoult D, Derrien-Courtel S, Fernandez C, Fredriksen S, Gevaert F, Gundersen H, Le Gal A, Lévêque L, Mieszkowska N, Norderhaug KM, Oliveira P, Puente A, Rico JM, Rinde E, Schubert H, Strain EM, Valero M, Viard F, Sousa-Pinto I (2016) Status, trends and drivers of kelp forests in Europe: An expert assessment. *Biodivers Conserv* 25:1319–1348.

Armitage CS, Husa V, Petelenz-Kurdziel EA, Sjøtun K (2017) Growth and competition in a warmer ocean: A field experiment with a non-native and two native habitat-building seaweeds. *Mar Ecol Prog Ser* 573:85–99.

Assis J, Araújo MB, Serrão EA (2018) Projected climate changes threaten ancient refugia of kelp forests in the North Atlantic. *Glob Change Biol* 24:e55–e66.

Assis J, Berecibar E, Claro B, Alberto F, Reed D, Raimondi P, Serrão EA (2017) Major shifts at the range edge of marine forests: The combined effects of climate changes and limited dispersal. *Sci Rep* 7:44348.

Atkinson MJ, Smith SV (1983) C:N:P ratios of benthic marine plants. *Limnol Oceanogr* 28:568–574.

Bartsch I, Paar M, Fredriksen S, Schwanitz M, Daniel C, Hop H, Wiencke C (2016) Changes in kelp forest biomass and depth distribution in Kongsfjorden, Svalbard, between 1996–1998 and 2012–2014 reflect Arctic warming. *Polar Biol* 39:2021–2036.

Bartsch I, Vogt J, Pehlke C, Hanelt D (2013) Prevailing sea surface temperatures inhibit summer reproduction of the kelp *Laminaria digitata* at Helgoland (North Sea). *J Phycol* 49:1061–1073.

Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schubert H, Schumann R, Valentin K, Weinberger F, Wiese J (2008) The genus *Laminaria sensu lato*: Recent insights and developments. *Eur J Phycol* 43:1–86.

Bekkby T, Moy FE (2011) Developing spatial models of sugar kelp (*Saccharina latissima*) potential distribution under natural conditions and areas of its disappearance in Skagerrak. *Estuar Coast Shelf Sci* 95:477–483.

Belseth E (2012) Eco-physiology of the Arctic kelp *Laminaria solidungula*. Master Thesis. Norwegian University of Science and Technology.

Bennett S, Duarte CM, Marbà N, Wernberg T (2019) Integrating within-species variation in thermal physiology into climate change ecology. *Philos Trans R Soc B* 374:20180550.

Bennett S, Wernberg T, Connell SD, Hobday AJ, Johnson CR, Poloczanska ES (2016) The 'Great Southern Reef': Social, ecological and economic value of Australia's neglected kelp forests. *Mar Freshw Res* 67:47–56.

Bennett S, Wernberg T, Joy BA, De Bettignies T, Campbell AH (2015) Central and rear-edge populations can be equally vulnerable to warming. *Nat Commun* 6:10280.

Bischof K, Buschbaum C, Fredriksen S, Gordillo FJL, Heinrich S, Jiménez C, Lütz C, Molis M, Roleda MY, Schwanitz M, Wiencke C (2019) Kelps and environmental changes in Kongsfjorden: Stress perception and responses. In: *The ecosystem of Kongsfjorden, Svalbard. Advances in polar ecology*. Hop H, Wiencke C (eds) Springer International, Published AG, Switzerland, Cham, p 373–422.

Bischof K, Hanelt D, Aguilera J, Karsten U, Vögele B, Sawall T, Wiencke C (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. *Mar Biol* 140:1097–1106.

Bischof K, Hanelt D, Tüg H, Karsten U, Brouwer PEM, Wiencke C (1998) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). *Polar Biol* 20:388–395.

Bluhm BA, Gradinger R (2008) Regional variability in food availability for Arctic marine mammals. *Ecol Appl (Suppl):*S77–S96.

Boersma M, Grüner N, Tasso Signorelli N, Montoro PE, Peck MA, Wiltshire KH (2016) Projecting effects of climate change on marine systems: Is the mean all that matters? *Proc R Soc B* 283:20152274.

Bolton JJ (2010) The biogeography of kelps (Laminariales, Phaeophyceae): A global analysis with new insights from recent advances in molecular phylogenetics. *Helgol Mar Res* 64:263–279.

Bolton JJ, Lüning K (1982) Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. *Mar Biol* 66:89–94.

Bonebrake TC, Brown CJ, Bell JD, Blanchard JL, Chauvenet A, Champion C, Chen IC, Clark TD, Colwell RK, Danielsen F, Dell AI, Donelson JM, Evengård B, Ferrier S, Frusher S, Garcia RA, Griffis RB, Hobday AJ, Jarzyna MA, Lee E, Lenoir J, Linnetved H, Martin VY, McCormack PC, McDonald J, McDonald-Madden E, Mitchell N, Mustonen T, Pandolfi JM, Pettorelli N, Possingham H, Pulsifer P, Reynolds M, Scheffers BR, Sorte CJB, Strugnell JM, Tuanmu MN, Twiname S, Vergés A, Villanueva C, Wapstra E, Wernberg T, Pecl GT (2018) Managing consequences of climate-driven species redistribution requires integration of ecology, conservation and social science. *Biol Rev* 93:284–305.

Britton D, Cornwall CE, Revill AT, Hurd CL, Johnson CR (2016) Ocean acidification reverses the positive effects of seawater pH fluctuations on growth and photosynthesis of the habitat-forming kelp, *Ecklonia radiata*. *Sci Rep* 6:26036.

Brown AD, Simpson JR (1972) Water relations of sugar-tolerant yeasts: The role of intracellular polyols. *J Gen Microbiol* 72:589–591.

Burdett HL, Wright H, Smale DA (2019) Photophysiological responses of canopy-forming kelp species to short-term acute warming. *Front Mar Sci* 6:516.

Camus C, Ballerino P, Rocio D, Olivera-Nappa Á, Leyton C, Buschmann AH (2016) Scaling up bioethanol production from the farmed brown macroalga *Macrocystis pyrifera* in Chile. *Biofuel Bioprod Biorefin* 10:673–685.

Casado-Amezúa P, Araújo R, Bárbara I, Bermejo R, Borja, Díez I, Fernández C, Gorostiaga JM, Guinda X, Hernández I, Juanes JA, Peña V, Peteiro C, Puente A, Quintana I, Tuya F, Viejo RM, Altamirano M, Gallardo T, Martínez B (2019) Distributional shifts of canopy-forming seaweeds from the Atlantic coast of Southern Europe. *Biodivers Conserv* 28:1151–1172.

Chapman ARO, Lindley JE (1980) Seasonal growth of *Laminaria solidungula* in the Canadian High Arctic in relation to irradiance and dissolved nutrient concentrations. *Mar Biol* 57:1–5.

Chapman ARO, Markham JW, Lüning K (1978) Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. *J Phycol* 14:195–198.

Christie H, Andersen GS, Bekkby T, Fagerli CW, Gitmark JK, Gundersen H, Rinde E (2019a) Shifts between sugar kelp and turf algae in Norway: Regime shifts or fluctuations between different opportunistic seaweed species? *Front Mar Sci* 6:72.

Christie H, Fredriksen S, Rinde E (1998) Regrowth of kelp and colonization of epiphyte and fauna community after kelp trawling at the coast of Norway. *Hydrobiologia* 375–376:49–58.

Christie H, Gundersen H, Rinde E, Filbee-Dexter K, Norderhaug KM, Pedersen T, Bekkby T, Gitmark JK, Fagerli CW (2019b) Can multitrophic interactions and ocean warming influence large-scale kelp recovery? *Ecol Evol* 9:1–16.

Christie H, Norderhaug KM, Fredriksen S (2009) Macrophytes as habitat for fauna. *Mar Ecol Prog Ser* 396:221–233.

Chung IK, Beardall J, Mehta S, Sahoo D, Stojkovic S (2011) Using marine macroalgae for carbon sequestration: A critical appraisal. *J Appl Phycol* 23:877–886.

Churchill FB (1974) William Johannsen and the genotype concept. *J Hist Biol* 7:5–30.

Conner JK, Hartl DL (2004) A primer of ecological genetics. Sinauer Associates Incorporated, Sunderland, USA.

Cottier F, Tverberg V, Inall M, Svendsen H, Nilsen F, Griffiths C (2005) Water mass modification in an Arctic fjord through cross-shelf exchange: The seasonal hydrography of Kongsfjorden, Svalbard. *J Geophys Res Ocean* 110:C12005.

Cottier FR, Nilsen F, Enall ME, Gerland S, Tverberg V, Svendsen H (2007) Wintertime warming of an Arctic shelf in response to large-scale atmospheric circulation. *Geophys Res Lett* 34:1–5.

Dalpadado P, Hop H, Rønning J, Pavlov V, Sperfeld E, Buchholz F, Rey A, Wold A (2016) Distribution and abundance of euphausiids and pelagic amphipods in Kongsfjorden, Isfjorden and Rijpfjorden (Svalbard) and changes in their relative importance as key prey in a warming marine ecosystem. *Polar Biol* 39:1765–1784.

Daniel RM, Dines M, Petach HH (1996) The denaturation and degradation of stable enzymes at high temperatures. *Biochem J* 317:1–11.

Davison IR (1987) Adaptation of photosynthesis in *Laminaria saccharina* (Phaeophyta) to changes in growth temperature. *J Phycol* 23:273–283.

Davison IR, Davison JO (1987) The effect of growth temperature on enzyme activities in the brown alga *Laminaria saccharina*. *Br Phycol J* 22:77–87.

Davison IR, Greene RM, Podolak EJ (1991) Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina*. *Mar Biol* 110:449–454.

Dayton PK (1985) Ecology of kelp communities. *Annu Rev Ecol Syst* 16:215–245.

Dillehay TD, Ramírez C, Pino M, Collins MB, Rossen J, Pino-Navarro JD (2008) Monte Verde: Seaweed, food, medicine, and the peopling of South America. *Science* 320:784–787.

Dring MJ (2006) Stress resistance and disease resistance in seaweeds: The role of reactive oxygen metabolism. *Adv Bot Res* 43:175–207.

Duarte CM, Wu J, Xiao X, Bruhn A, Krause-Jensen D (2017) Can seaweed farming play a role in climate change mitigation and adaptation? *Front Mar Sci* 4:100.

Dunton KH (1985) Growth of dark-exposed *Laminaria saccharina* (L.) Lamour. and *Laminaria solidungula* J. Ag. (Laminariales:Phaeophyta) in the Alaskan Beaufort Sea. *J Exp Mar Bio Ecol* 94:181–189.

Dunton KH, Reimnitz E, Schonberg S (1982) An Arctic kelp community in the Alaskan Beaufort Sea. *Arctic* 35:465–484.

Dunton KH, Schell DM (1986) Seasonal carbon budget and growth of *Laminaria solidungula* in the Alaskan High Arctic. *Mar Ecol* 31:57–66.

Endo H, Suehiro K, Gao X, Agatsuma Y (2017) Interactive effects of elevated summer temperature, nutrient availability, and irradiance on growth and chemical compositions of juvenile kelp, *Eisenia bicyclis*. *Phycol Res* 65:118–126.

Eriksson BK, Johansson G, Snoeijs P (2002) Long-term changes in the macroalgal vegetation of the inner Gullmar Fjord, Swedish Skagerrak coast. *J Phycol* 38:284–296.

Erlandson JM, Graham MH, Bourque BJ, Corbett D, Estes JA, Steneck RS (2007) The kelp highway hypothesis: Marine ecology, the coastal migration theory, and the peopling of the Americas. *J Isl Coast Archaeol* 2:161–174.

Fernandes F, Barbosa M, Oliveira AP, Azevedo IC, Sousa-Pinto I, Valentão P, Andrade PB (2016) The pigments of kelps (Ochrophyta) as part of the flexible response to highly variable marine environments. *J Appl Phycol* 28:3689–3696.

Fernández PA, Gaitán-Espitia JD, Leal PP, Schmid M, Revill AT, Hurd CL (2020) Nitrogen sufficiency enhances thermal tolerance in habitat-forming kelp: Implications for acclimation under thermal stress. *Sci Rep* 10:3186.

Fernández PA, Roleda MY, Hurd CL (2015) Effects of ocean acidification on the photosynthetic performance, carbonic anhydrase activity and growth of the giant kelp *Macrocystis pyrifera*. *Photosynth Res* 124:293–304.

Filbee-Dexter K (2020) Ocean forests hold unique solutions to our current environmental crisis. *One Earth* 2:398–401.

Filbee-Dexter K, Scheibling RE (2014) Sea urchin barrens as alternative stable states of collapsed kelp ecosystems. *Mar Ecol Prog Ser* 495:1–25.

Filbee-Dexter K, Smajdor A (2019) Ethics of assisted evolution in marine conservation. *Front Mar Sci* 6:20.

Filbee-Dexter K, Wernberg T (2018) Rise of turfs: A new battlefront for globally declining kelp forests. *Bioscience* 68:64–76.

Filbee-Dexter K, Wernberg T, Fredriksen S, Norderhaug KM, Pedersen MF (2019) Arctic kelp forests: Diversity, resilience and future. *Glob Planet Change* 172:1–14.

Filbee-Dexter K, Wernberg T, Grace SP, Thormar J, Fredriksen S, Narvaez CN, Feehan CJ, Norderhaug KM (2020) Marine heatwaves and the collapse of marginal North Atlantic kelp forests. *Sci Rep* 10:13388.

Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgol Meeresunters* 34:15–29.

Franco JN, Tuya F, Bertocci I, Rodríguez L, Martínez B, Sousa-Pinto I, Arenas F (2018) The 'golden kelp' *Laminaria ochroleuca* under global change: Integrating multiple eco-physiological responses with species distribution models. *J Ecol* 106:47–58.

Franklin L, Forster R (1997) The changing irradiance environment: Consequences for marine macrophyte physiology, productivity and ecology. *Eur J Phycol* 32:207–232.

Fraser CI (2012) Short review: Is bull-kelp kelp? The role of common names in science. *New Zeal J Mar Freshw Res* 46:279–284.

Fredersdorf J, Müller R, Becker S, Wiencke C, Bischof K (2009) Interactive effects of radiation, temperature and salinity on different life history stages of the Arctic kelp *Alaria esculenta* (Phaeophyceae). *Oecologia* 160:483–492.

Froehlich HE, Afflerbach JC, Frazier M, Halpern BS (2019) Blue growth potential to mitigate climate change through seaweed offsetting. *Curr Biol* 29:3087–3093.

Frölicher TL, Fischer EM, Gruber N (2018) Marine heatwaves under global warming. *Nature* 560:360–364.

Gao X, Endo H, Nagaki M, Agatsuma Y (2016) Growth and survival of juvenile sporophytes of the kelp *Ecklonia cava* in response to different nitrogen and temperature regimes. *Fish Sci* 82:623–629.

Gaylord B, Denny MW, Koehl MAR (2008) Flow forces on seaweeds: Field evidence for roles of wave impingement and organism inertia. *Biol Bull* 215:295–308.

Gerard VA (1988) Ecotypic differentiation in light-related traits of the kelp *Laminaria saccharina*. *Mar Biol* 97:25–36.

Gerard VA (1990) Ecotypic differentiation in the kelp *Laminaria saccharina*: Phase-specific adaptation in a complex life cycle. *Mar Biol* 107:519–528.

Gerard VA, Du Bois KR (1988) Temperature ecotypes near the southern boundary of the kelp *Laminaria saccharina*. *Mar Biol* 97:575–580.

Gerard VA, DuBois K, Greene R (1987) Growth responses of two *Laminaria saccharina* populations to environmental variation. *Hydrobiologia* 151/152:229–232.

Gerard VA (1997) The role of nitrogen nutrition in high-temperature tolerance of the kelp, *Laminaria saccharina* (Chromophyta). *J Phycol* 33:800–810.

Gevaert F, Davoult D, Creach A, Kling R, Janquin M-A, Seuront L, Lemoine Y (2001) Carbon and nitrogen content of *Laminaria saccharina* in the eastern English Channel: Biometrics and seasonal variations. *J Mar Biol Assoc U K* 81:727–734.

Gómez I, Español S, Véliz K, Huovinen P (2016) Spatial distribution of phlorotannins and its relationship with photosynthetic UV tolerance and allocation of storage carbohydrates in blades of the kelp *Lessonia spicata*. *Mar Biol* 163:110.

Gordillo FJL, Aguilera J, Jiménez C (2006) The response of nutrient assimilation and biochemical composition of Arctic seaweeds to a nutrient input in summer. *J Exp Bot* 57:2661–2671.

Gordillo FJL, Dring MJ, Savidge G (2002) Nitrate and phosphate uptake characteristics of three species of brown algae cultured at low salinity. *Mar Ecol Prog Ser* 234:111–118.

Goss R, Jakob T (2010) Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynth Res* 106:103–122.

Graiff A, Ruth W, Kragl U, Karsten U (2016) Chemical characterization and quantification of the brown algal storage compound laminarin – A new methodological approach. *J Appl Phycol* 28:533–543.

Guzinski J, Mauger S, Cock JM, Valero M (2016) Characterization of newly developed expressed sequence tag-derived microsatellite markers revealed low genetic diversity within and low connectivity between European *Saccharina latissima* populations. *J Appl Phycol* 28:3057–3070.

Guzinski J, Ruggeri P, Ballenghien M, Mauger S, Jacquemin B, Jollivet C, Coudret J, Jaugeon L, Destombe C, Valero M (2020) Seascape genomics of the sugar kelp *Saccharina latissima* along the north eastern Atlantic latitudinal gradient. *Genes* 11:1503.

Gylle AM, Nygard CA, Ekelund NGA (2009) Desiccation and salinity effects on marine and brackish *Fucus vesiculosus* L. (Phaeophyceae). *Phycologia* 48:156–164.

Hafting JT, Craigie JS, Stengel DB, Loureiro RR, Buschmann AH, Yarish C, Edwards MD, Critchley AT (2015) Prospects and challenges for industrial production of seaweed bioactives. *J Phycol* 51:821–837.

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: The rear edge matters. *Ecol Lett* 8:461–467.

Hanelt D, Tüg H, Bischof K, Groß C, Lippert H, Sawall T, Wiencke C (2001) Light regime in an Arctic fjord: A study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Mar Biol* 138:649–658.

Harley CDG, Anderson KM, Demes KW, Jorve JP, Kordas RL, Coyle TA, Graham MH (2012) Effects of climate change on global seaweed communities. *J Phycol* 48:1064–1078.

Harley CDG, Hughes AR, Kristin M, Miner BG, Sorte CJB, Thornber CS, Rodríguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine systems. *Ecol Lett* 9:228–241.

Harris RMB, Beaumont LJ, Vance TR, Tozer CR, Remenyi TA, Perkins-Kirkpatrick SE, Mitchell PJ, Nicotra AB, McGregor S, Andrew NR, Letnic M, Kearney MR, Wernberg T, Hutley LB, Chambers LE, Fletcher MS, Keatley MR, Woodward CA, Williamson G, Duke NC, Bowman DMJS (2018) Biological responses to the press and pulse of climate trends and extreme events. *Nat Clim Change* 8:579–587.

Hart MW, Scheibling RE (1988) Heat waves, baby booms, and the destruction of kelp beds by sea urchins. *Mar Biol* 99:167–176.

Hayashida H, Matear RJ, Strutton PG, Zhang X (2020) Insights into projected changes in marine heatwaves from a high-resolution ocean circulation model. *Nat Commun* 11:4352.

Heinrich S, Valentin K, Frickenhaus S, Wiencke C (2016) Origin matters – Comparative transcriptomics in *Saccharina latissima* (Phaeophyceae). *J Exp Mar Bio Ecol* 476:22–30.

Heinrich S, Valentin K, Frickenhaus S, Wiencke C (2015) Temperature and light interactively modulate gene expression in *Saccharina latissima* (Phaeophyceae). *J Phycol* 51:93–108.

Henley WJ, Dunton KH (1995) A seasonal comparison of carbon, nitrogen, and pigment content in *Laminaria solidungula* and *L. saccharina* (Phaeophyta) in the Alaskan Arctic. *J Phycol* 31:325–331.

Henley WJ, Dunton KH (1997) Effects of nitrogen supply and continuous darkness on growth and photosynthesis of the arctic kelp *Laminaria solidungula*. *Limnol Oceanogr* 42:209–216.

Hobday AJ, Alexander L V., Perkins SE, Smale DA, Straub SC, Oliver ECJ, Benthuysen JA, Burrows MT, Donat MG, Feng M, Holbrook NJ, Moore PJ, Scannell HA, Sen Gupta A, Wernberg T (2016) A hierarchical approach to defining marine heatwaves. *Prog Oceanogr* 141:227–238.

van den Hoek C, Mann DG, Jahns HM (1995) Algae – An introduction to phycology. Cambridge University Press, Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo, New Dehli.

Holderegger R, Kamm U, Gugerli F (2006) Adaptive vs. neutral genetic diversity: Implications for landscape genetics. *Landsc Ecol* 21:797–807.

Hop H, Wiencke C, Vögele B, Kovaltchouk NA (2012) Species composition, zonation, and biomass of marine benthic macroalgae in Kongsfjorden, Svalbard. *Bot Mar* 55:399–414.

Hurd CL, Harrison PJ, Bischof K, Lobban CS (2014) Seaweed ecology and physiology, Second Edi. Cambridge University Press, Cambridge.

Iñiguez C, Carmona R, Lorenzo MR, Niell FX, Wiencke C, Gordillo FJL (2016) Increased temperature, rather than elevated CO_2, modulates the carbon assimilation of the Arctic kelps *Saccharina latissima* and *Laminaria solidungula*. *Mar Biol* 163:248.

IPCC (2019) IPCC Special report on the ocean and cryosphere in a changing climate. Pörtner H-O, Roberts DC, Masson-Delmotte V, Zhai P, Tignor M, Poloczanska E, Mintenbeck K, Alegría A, Nicolai M, Okem A, Petzold J, Rama B, Weyer NM (eds) Intergovernmental Panel on Climate Change.

Iwamoto K, Shiraiwa Y (2005) Salt-regulated mannitol metabolism in algae. *Mar Biotechnol* 7:407–415.

Jennings DB, Ehrenshaft M, Mason Pharr D, Williamson JD (1998) Roles for mannitol and mannitol dehydrogenase in active oxygen-mediated plant defense. *Proc Natl Acad Sci U S A* 95:15129–15133.

Jiménez C, Niell FX (1991) Influence of temperature and salinity on carbon and nitrogen content in *Dunaliella viridis* teodoresco under nitrogen sufficiency. *Bioresour Technol* 38:91–94.

de Jong G (2005) Evolution of phenotypic plasticity: Patterns of plasticity and the emergence of ecotypes. *New Phytol* 166:101–118.

Jueterbock A, Minne AJP, Cock JM, Coleman MA, Wernberg T, Scheschonk L, Rautenberger R, Hu Z-M (2021) Priming of marine macrophytes for enhanced restoration success and food security in future oceans. *Front Mar Sci* 8:658485.

Kain (Jones) JM (1989) The seasons in the subtidal. *Br Phycol J* 24:203–215.

Karsten U (2007) Salinity tolerance of Arctic kelps from Spitsbergen. *Phycol Res* 55:257–262.

Karsten U (2012) Seaweed acclimation to salinity and desiccation stress. In: *Seaweed Biology – Novel insights into ecophysiology, ecology and utilization*. Wiencke C, Bischof K (eds) Springer-Verlag Berlin Heidelberg, p 87–107.

Karsten U, Dummermuth A, Hoyer K, Wiencke C (2003) Interactive effects of ultraviolet radiation and salinity on the ecophysiology of two Arctic red algae from shallow waters. *Polar Biol* 26:249–258.

Kautsky L, Kautsky N (2000) The Baltic Sea, including Bothnian Sea and Bothnian Bay. In: *Seas at the millennium: An environmental evaluation*. Sheppard CRC (ed) Elsevier Science Ltd.

Kelly MW, Sanford E, Grosberg RK (2012) Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proc R Soc B Biol Sci* 279:349–356.

Kerrison PD, Stanley MS, Edwards MD, Black KD, Hughes AD (2015) The cultivation of European kelp for bioenergy: Site and species selection. *Biomass Bioenergy* 80:229–242.

Kim JK, Yarish C, Hwang EK, Park M, Kim Y (2017) Seaweed aquaculture: Cultivation technologies, challenges and its ecosystem services. *Algae* 32:1–13.

King NG, Mckeown NJ, Smale DA, Moore PJ (2018a) The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. *Ecography* 41:1469–1484.

King NG, McKeown NJ, Smale DA, Wilcockson DC, Hoelters L, Groves EA, Stamp T, Moore PJ (2019) Evidence for different thermal ecotypes in range centre and trailing edge kelp populations. *J Exp Mar Bio Ecol* 514–515:10–17.

King NG, Wilcockson DC, Webster R, Smale DA, Hoelters LS, Moore PJ (2018b) Cumulative stress restricts niche filling potential of habitat-forming kelps in a future climate. *Funct Ecol* 32:288–299.

Kirst GO (1989) Salinity tolerance of eukaryotic marine algae. *Annu Rev Plant Physiol Plant Mol Biol* 40:21–53.

Korb RE, Gerard VA (2000) Effects of concurrent low temperature and low nitrogen supply on polar and temperate seaweeds. *Mar Ecol Prog Ser* 198:73–82.

Krause-Jensen D, Archambault P, Assis J, Bartsch I, Bischof K, Filbee-dexter K, Dunton KH, Maximova O, Ragnarsdóttir SB, Sejr MK, Simakova U, Spiridonov V, Wegeberg S, Winding MHS, Duarte CM (2020) Imprint of climate change on Pan-Arctic marine vegetation. *Front Mar Sci* 7:1–27.

Krause-Jensen D, Duarte CM (2014) Expansion of vegetated coastal ecosystems in the future Arctic. *Front Mar Sci* 1:77.

Krause-Jensen D, Duarte CM (2016) Substantial role of macroalgae in marine carbon sequestration. *Nat Geosci* 9:737–742.

Krause-Jensen D, Marbà N, Olesen B, Sejr MK, Christensen PB, Rodrigues J, Renaud PE, Balsby TJS, Rysgaard S (2012) Seasonal sea ice cover as principal driver of spatial and temporal variation in depth extension and annual production of kelp in Greenland. *Glob Change Biol* 18:2981–2994.

Krumhansl KA, Okamoto DK, Rassweiler A, Novak M, Bolton JJ, Cavanaugh KC, Connell SD, Johnson CR, Konar B, Ling SD, Micheli F, Norderhaug KM, Pérez-Matus A, Sousa-Pinto I, Reed DC, Salomon AK, Shears NT, Wernberg T, Anderson RJ, Barrett NS, Buschmann AH, Carr MH, Caselle JE, Derrien-Courtel S, Edgar GJ, Edwards M, Estes JA, Goodwin C, Kenner MC, Kushner DJ, Moy FE, Nunn J, Steneck RS, Vásquez J, Watson J, Witman JD, Byrnes JEK (2016) Global patterns of kelp forest change over the past half-century. *Proc Natl Acad Sci* 113:13785–13790.

Laeseke P, Bartsch I, Bischof K (2019) Effects of kelp canopy on underwater light climate and viability of brown algal spores in Kongsfjorden (Spitsbergen). *Polar Biol* 42:1511–1527.

Lane CE, Mayes C, Druehl LD, Saunders GW (2006) A multi-gene molecular investigation of the kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. *J Phycol* 42:493–512.

Leroi AM, Bennett AF, Lenski RE (1994) Temperature acclimation and competitive fitness: An experimental test of the beneficial acclimation assumption. *Proc Natl Acad Sci U S A* 91:1917–1921.

Leshem YY, Kuiper PJC (1996) Is there a GAS (general adaptation syndrome) response to various types of environmental stress? *Biol Plant* 38:1–18.

Li H, Monteiro C, Heinrich S, Bartsch I, Valentin K, Harms L, Glöckner G, Corre E, Bischof K (2020a) Responses of the kelp *Saccharina latissima* (Phaeophyceae) to the warming Arctic: From physiology to transcriptomics. *Physiol Plant* 168:5–26.

Li H, Scheschonk L, Heinrich S, Valentin K, Harms L, Glöckner G, Corre E, Bischof K, Kennedy F (2020b) Transcriptomic responses to darkness and the survival strategy of the kelp *Saccharina latissima* in the early polar night. *Front Mar Sci* 7:592033.

Liesner D (2020) Thermal trait variability of the kelp *Laminaria digitata* across populations and life cycle stages. Doctoral Thesis. University of Bremen.

Liesner D, Fouqueau L, Valero M, Roleda MY, Pearson GA, Bischof K, Valentin K, Bartsch I (2020a) Heat stress responses and population genetics of the kelp *Laminaria digitata* (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations. *Ecol Evol* 10: 9144–9177.

Liesner D, Shama LNS, Diehl N, Valentin K, Bartsch I (2020b) Thermal plasticity of the kelp *Laminaria digitata* (Phaeophyceae) across life cycle stages reveals the importance of cold seasons for marine forests. *Front Mar Sci* 7:456.

Lima FP, Wethey DS (2012) Three decades of high-resolution coastal sea surface temperatures reveal more than warming. *Nat Commun* 3:704.

Lind AC, Konar B (2017) Effects of abiotic stressors on kelp early life-history stages. *Algae* 32:223–233.

Lotze HK, Worm B (2002) Complex interactions of climatic and ecological controls on macroalgal recruitment. *Limnol Oceanogr* 47:1734–1741.

Lourenço CR, Zardi GI, McQuaid CD, Serrão EA, Pearson GA, Jacinto R, Nicastro KR (2016) Upwelling areas as climate change refugia for the distribution and genetic diversity of a marine macroalga. *J Biogeogr* 43:1595–1607.

Løvås SM, Tørum A (2001) Effect of the kelp *Laminaria hyperborea* upon sand dune erosion and water particle velocities. *Coast Eng* 44:37–63.

Lüning K (1975) Kreuzungsexperimente an *Laminaria saccharina* von Helgoland und von der Isle of Man. *Helgol Wiss Meeresunters* 27:108–114.

Lüning K (1988) Photoperiodic control of sorus formation in the brown alga *Laminaria saccharina*. *Mar Ecol Prog Ser* 45:137–144.

Lüning K (1990) Seaweeds – Their environment, biogeography, and ecophysiology. Yarish C, Kirkman H (eds) John Wiley & Sons, Inc.

Lüning K (1984) Temperature tolerance and biogeography of seaweeds: The marine algal flora of Helgoland (North Sea) as an example. *Helgol Meeresunters* 28:305–317.

Luttikhuizen PC, van den Heuvel FHM, Rebours C, Witte HJ, van Bleijswijk JDL, Timmermans K (2018) Strong population structure but no equilibrium yet: Genetic connectivity and phylogeography in the kelp *Saccharina latissima* (Laminariales, Phaeophyta). *Ecol Evol* 8:4265–4277.

Mandal SK, Ajay G, Monisha N, Malarvizhi J, Temkar G, Mantri VA (2015) Differential response of varying temperature and salinity regimes on nutrient uptake of drifting fragments of *Kappaphycus alvarezii*: Implication on survival and growth. *J Appl Phycol* 27:1571–1581.

Mann KH (1973) Seaweeds: Their productivity and stategy for growth. *Science* 182:975–981.

Martins CDL, Lhullier C, Ramlov F, Simonassi JC, Gouvea LP, Noernberg M, Maraschin M, Colepicolo P, Hall-Spencer JM, Horta PA (2014) Seaweed chemical diversity: An additional and efficient tool for coastal evaluation. *J Appl Phycol* 26:2037–2045.

Martins N, Pearson GA, Bernard J, Serrão EA, Bartsch I (2020) Thermal traits for reproduction and recruitment differ between Arctic and Atlantic kelp *Laminaria digitata*. *PLoS One* 15:e0235388.

Martins N, Tanttu H, Pearson GA, Serrão EA, Bartsch I (2017) Interactions of daylength, temperature and nutrients affect thresholds for life stage transitions in the kelp *Laminaria digitata* (Phaeophyceae). *Bot Mar* 60:109–121.

Maturilli M, Herber A, König-Langlo G (2013) Climatology and time series of surface meteorology in Ny-Ålesund, Svalbard. *Earth Syst Sci Data* 5:155–163.

McPherson ML, Finger DJI, Houskeeper HF, Bell TW, Carr MH, Rogers-Bennett L, Kudela RM (2021) Large-scale shift in the structure of a kelp forest ecosystem co-occurs with an epizootic and marine heatwave. *Commun Biol* 4:298.

Meehl GA, Tebaldi C (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. *Science* 305:994–997.

Meredith M, Sommerkorn M, Cassotta S, Derksen S, Ekaykin A, Hollowed A, Kofinas G, Mackintosh A, Melbourne-Thomas J, Muelbert MMC, Ottersen G, Pritchard H, Schuur EAG (2019) Polar Regions. In: *IPCC Special report on the ocean and cryosphere in a changing climate*. Pörtner H-O, Roberts DC, Masson-Delmotte V, Zhai P, Tignor M, Poloczanska E, Mintenbeck K, Alegría A, Nicolai M, Okem A, Petzold J, Rama B, Weyer NM (eds) p 203–320.

Mineur F, Arenas F, Assis J, Davies AJ, Engelen AH, Fernandes F, Malta E jan, Thibaut T, Van Nguyen T, Vaz-Pinto F, Vranken S, Serrão EA, De Clerck O (2015) European seaweeds under pressure: Consequences for communities and ecosystem functioning. *J Sea Res* 98:91–108.

Mac Monagail M, Cornish L, Morrison L, Araújo R, Critchley AT (2017) Sustainable harvesting of wild seaweed resources. *Eur J Phycol* 52:371–390.

Monteiro C (2020) Transciptional bases of acclimation in the kelp, *Saccharina latissima*, from gametophytes to sporophytes. Doctoral Thesis. Sorbonne Université / University of Bremen.

Monteiro C, Heinrich S, Bartsch I, Valentin K, Corre E, Collén J, Harms L, Glöckner G, Bischof K (2019) Temperature modulates sex-biased gene expression in the gametophytes of the kelp *Saccharina latissima*. *Front Mar Sci* 6:769.

Monteiro C, Li H, Diehl N, Collén J, Heinrich S, Bischof K, Bartsch I (2021) Modulation of physiological performance by temperature and salinity in the sugar kelp *Saccharina latissima*. *Phycol Res* 69:48–57.

Monteiro JP, Rey F, Melo T, Moreira ASP, Arbona JF, Skjermo J, Forbord S, Funderud J, Raposo D, Kerrison PD, Perrineau MM, Gachon C, Domingues P, Calado R, Domingues MR (2020) The unique lipidomic signatures of *Saccharina latissima* can be used to pinpoint their geographic origin. *Biomolecules* 10:107.

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Huner NPA (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiol Mol Biol Rev* 70:222–252.

Mota CF, Engelen AH, Serrão EA, Pearson GA (2015) Some don't like it hot: Microhabitat-dependent thermal and water stresses in a trailing edge population. *Funct Ecol* 29:640–649.

Moy FE, Christie H (2012) Large-scale shift from sugar kelp (*Saccharina latissima*) to ephemeral algae along the south and west coast of Norway. *Mar Biol Res* 8:309–321.

Müller R, Desel C, Steinhoff FS, Wiencke C, Bischof K (2012) UV-radiation and elevated temperatures induce formation of reactive oxygen species in gametophytes of cold-temperate/Arctic kelps (Laminariales, Phaeophyceae). *Phycol Res* 60:27–36.

Müller R, Laepple T, Bartsch I, Wiencke C (2009) Impact of oceanic warming on the distribution of seaweeds in polar and cold-temperate waters. *Bot Mar* 52:617–638.

Müller R, Wiencke C, Bischof K (2008) Interactive effects of UV radiation and temperature on microstages of Laminariales (Phaeophyceae) from the Arctic and North Sea. *Clim Res* 37:203–213.

Neiva J, Paulino C, Nielsen MM, Krause-Jensen D, Saunders GW, Assis J, Bárbara I, Tamigneaux É, Gouveia L, Aires T, Marbà N, Bruhn A, Pearson GA, Serraõ EA (2018) Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissima*. *Sci Rep* 8:1112.

Neiva J, Serrão EA, Paulino C, Gouveia L, Want A, Tamigneaux É, Ballenghien M, Mauger S, Fouqueau L, Engel-Gautier C, Destombe C, Valero M (2020) Genetic structure of amphi-Atlantic *Laminaria digitata* (Laminariales, Phaeophyceae) reveals a unique range-edge gene pool and suggests post-glacial colonization of the NW Atlantic. *Eur J Phycol* 55:517–528.

Nellemann C, Corcoran E, Duarte CM, Valdés L, De Young C, Fonseca L, Grimsditch G (2009) Blue carbon. A rapid response assessment. Nellemann C, Corcoran E, Duarte CM, Valdés L, De Young C, Fonseca L, Grimsditch G (eds). United Nations Environment Programme, GRID-Arendal, www.grida.no, Arendal, Norway.

Nepper-Davidsen J, Andersen D, Pedersen M (2019) Exposure to simulated heatwave scenarios causes long-term reductions in performance in *Saccharina latissima*. *Mar Ecol Prog Ser* 630:25–39.

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. *Trends Plant Sci* 15:684–692.

Nielsen MM, Krause-Jensen D, Olesen B, Thinggaard R, Christensen PB, Bruhn A (2014) Growth dynamics of *Saccharina latissima* (Laminariales, Phaeophyceae) in Aarhus Bay, Denmark, and along the species' distribution range. *Mar Biol* 161:2011–2022.

Nielsen MM, Manns D, D'Este M, Krause-Jensen D, Rasmussen MB, Larsen MM, Alvarado-Morales M, Angelidaki I, Bruhn A (2016a) Variation in biochemical composition of *Saccharina latissima* and *Laminaria digitata* along an estuarine salinity gradient in inner Danish waters. *Algal Res* 13:235–245.

Nielsen MM, Paulino C, Neiva J, Krause-Jensen D, Bruhn A, Serrão EA (2016b) Genetic diversity of *Saccharina latissima* (Phaeophyceae) along a salinity gradient in the North Sea-Baltic Sea transition zone. *J Phycol* 52:523–531.

Olischläger M, Bartsch I, Gutow L, Wiencke C (2012) Effects of ocean acidification on different life-cycle stages of the kelp *Laminaria hyperborea* (Phaeophyceae). *Bot Mar* 55:511–525.

Olischläger M, Iñiguez C, Gordillo FJL, Wiencke C (2014) Biochemical composition of temperate and Arctic populations of *Saccharina latissima* after exposure to increased pCO_2 and temperature reveals ecotypic variation. *Planta* 240:1213–1224.

Olischläger M, Iñiguez C, Koch K, Wiencke C, Gordillo FJL (2017) Increased pCO_2 and temperature reveal ecotypic differences in growth and photosynthetic performance of temperate and Arctic populations of *Saccharina latissima*. *Planta* 245:119–136.

Oliver ECJ, Benthuysen JA, Darmaraki S, Donat MG, Hobday AJ, Holbrook NJ, Schlegel RW, Sen Gupta A (2021) Marine Heatwaves. *Ann Rev Mar Sci* 13:313–342.

Oliver ECJ, Donat MG, Burrows MT, Moore PJ, Smale DA, Alexander L V., Benthuysen JA, Feng M, Sen Gupta A, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell HA, Straub SC, Wernberg T (2018) Longer and more frequent marine heatwaves over the past century. *Nat Commun* 9:1324.

Orr HA (2009) Fitness and its role in evolutionary genetics. *Nat Rev Genet* 10:531–539.

Ortega A, Geraldi NR, Alam I, Kamau AA, Acinas SG, Logares R, Gasol JM, Massana R, Krause-Jensen D, Duarte CM (2019) Important contribution of macroalgae to oceanic carbon sequestration. *Nat Geosci* 12:748–754.

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annu Rev Ecol Evol Syst* 37:637–671.

Pearse JS, Hines AH (1979) Expansion of a central California kelp forest following the mass mortality of sea urchins. *Mar Biol* 51:83–91.

Pehlke C, Bartsch I (2008) Changes in depth distribution and biomass of sublittoral seaweeds at Helgoland (North Sea) between 1970 and 2005. *Clim Res* 37:135–147.

Pereira TR, Engelen AH, Pearson GA, Valero M, Serrão EA (2015) Response of kelps from different latitudes to consecutive heat shock. *J Exp Mar Bio Ecol* 463:57–62.

Peteiro C, Sánchez N (2012) Comparing salinity tolerance in early stages of the sporophytes of a non-indigenous kelp (*Undaria pinnatifida*) and a native kelp (*Saccharina latissima). Russ J Mar Biol* 38:197–200.

Peters KJ, Amsler CD, Amsler MO, McClintock JB, Dunbar RB, Baker BJ (2005) A comparative analysis of the nutritional and elemental composition of macroalgae from the western Antarctic Peninsula. *Phycologia* 44:453–463.

Pfister CA, Van Alstyne KL (2003) An experimental assessment of the effects of nutrient enhancement on the intertidal kelp *Hedophyllum sessile* (Laminarales, Phaeophyceae). *J Phycol* 39:285–290.

Pfister CA, Altabet MA, Weigel BL (2019) Kelp beds and their local effects on seawater chemistry, productivity, and microbial communities. *Ecology* 100:e02798.

Pinho D, Bertocci I, Arenas F, Franco JN, Jacinto D, Castro JJ, Vieira R, Sousa-Pinto I, Wernberg T, Tuya F (2016) Spatial and temporal variation of kelp forests and associated macroalgal assemblages along the Portuguese coast. *Mar Freshw Res* 67:113–122.

Pörtner HO, Farrell AP (2008) Physiology and climate change. *Science* 322:690–692.

Provost EJ, Kelaher BP, Dworjanyn SA, Russell BD, Connell SD, Ghedini G, Gillanders BM, Figueira WI, Coleman MA (2017) Climate-driven disparities among ecological interactions threaten kelp forest persistence. *Glob Change Biol* 23:353–361.

Raybaud V, Beaugrand G, Goberville E, Delebecq G, Destombe C, Valero M, Davoult D, Morin P, Gevaert F (2013) Decline in kelp in West Europe and climate. *PLoS One* 8:e66044.

Reed TE, Schindler DE, Waples RS (2011) Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conserv Biol* 25:56–63.

Rogers-Bennett L, Catton CA (2019) Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Sci Rep* 9:15050.

Rokkan Iversen K, Seuthe L (2011) Seasonal microbial processes in a high-latitude fjord (Kongsfjorden, Svalbard): I. Heterotrophic bacteria, picoplankton and nanoflagellates. *Polar Biol* 34:731–749.

Roleda MY (2009) Photosynthetic response of Arctic kelp zoospores exposed to radiation and thermal stress. *Photochem Photobiol Sci* 8:1302–1312.

Roleda MY (2016) Stress physiology and reproductive phenology of Arctic endemic kelp *Laminaria solidungula* J. Agardh. *Polar Biol* 39:1967–1977.

Roleda MY, Dethleff D (2011) Storm-generated sediment deposition on rocky shores: Simulating burial effects on the physiology and morphology of *Saccharina latissima* sporophytes. *Mar Biol Res* 7:213–223.

Roleda MY, Dethleff D, Wiencke C (2008) Transient sediment load on blades of Arctic *Saccharina latissima* can mitigate UV radiation effect on photosynthesis. *Polar Biol* 31:765–769.

Roleda MY, Hurd CL (2019) Seaweed nutrient physiology: Application of concepts to aquaculture and bioremediation. *Phycologia* 58:552–562.

Roleda MY, Morris JN, McGraw CM, Hurd CL (2012) Ocean acidification and seaweed reproduction: Increased CO_2 ameliorates the negative effect of lowered pH on meiospore germination in the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae). *Glob Change Biol* 18:854–864.

Rothäusler E, Gómez I, Karsten U, Tala F, Thiel M (2011) Physiological acclimation of floating *Macrocystis pyrifera* to temperature and irradiance ensures long-term persistence at the sea surface at mid-latitudes. *J Exp Mar Bio Ecol* 405:33–41.

Rothman MD, Mattio L, Anderson RJ, Bolton JJ (2017) A phylogeographic investigation of the kelp genus *Laminaria* (Laminariales, Phaeophyceae), with emphasis on the South Atlantic Ocean. *J Phycol* 53:778–789.

Saha M, Barboza FR, Somerfield PJ, Al-Janabi B, Beck M, Brakel J, Ito M, Pansch C, Nascimento-Schulze JC, Jakobsson Thor S, Weinberger F, Sawall Y (2020) Response of foundation macrophytes to near-natural simulated marine heatwaves. *Glob Change Biol* 26:417–430.

Scheschonk L, Becker S, Hehemann JH, Diehl N, Karsten U, Bischof K (2019) Arctic kelp eco-physiology during the polar night in the face of global warming: A crucial role for laminarin. *Mar Ecol Prog Ser* 611:59–74.

Schiel DR, Steinbeck JR, Foster MS (2004) Ten years of induced ocean warming causes comprehensive changes in marine benthic communities. *Ecology* 85:1833–1839.

Schiener P, Black KD, Stanley MS, Green DH (2015) The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta. J Appl Phycol* 27:363–373.

Seymour RJ, Tegner MJ, Dayton PK, Parnell PE (1989) Storm wave induced mortality of giant kelp, *Macrocystis pyrifera*, in Southern California. *Estuar Coast Shelf Sci* 28:277–292.

Shen B, Jensen RG, Bohnert HJ (1997) Mannitol protects against oxidation by hydroxyl radicals. *Plant Physiol* 115:527–532.

Simonson EJ, Metaxas A, Scheibling RE (2015a) Kelp in hot water: II. Effects of warming seawater temperature on kelp quality as a food source and settlement substrate. *Mar Ecol Prog Ser* 537:105–119.

Simonson EJ, Scheibling RE, Metaxas A (2015b) Kelp in hot water: I. Warming seawater temperature induces weakening and loss of kelp tissue. *Mar Ecol Prog Ser* 537:89–104.

Sjøtun K (1993) Seasonal lamina growth in two age groups of *Laminaria saccharina* (L.) Lamour. in western Norway. *Bot Mar* 36:433–442.

Smale DA (2020) Impacts of ocean warming on kelp forest ecosystems. *New Phytol* 225:1447–1454.

Smale DA, Wernberg T, Oliver ECJ, Thomsen M, Harvey BP, Straub SC, Burrows MT, Alexander L V., Benthuysen JA, Donat MG, Feng M, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell HA, Sen Gupta A, Payne BL, Moore PJ (2019) Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nat Clim Change* 9:306–312.

Springer K, Lütz C, Lütz-Meindl U, Wendt A, Bischof K (2017) Hyposaline conditions affect UV susceptibility in the Arctic kelp *Alaria esculenta* (Phaeophyceae). *Phycologia* 56:675–685.

Spurkland T, Iken K (2011a) Kelp bed dynamics in estuarine environments in subarctic Alaska. *J Coast Res* 27:133–143.

Spurkland T, Iken K (2011b) Salinity and irradiance effects on growth and maximum photosynthetic quantum yield in subarctic *Saccharina latissima* (Laminariales, Laminariaceae). *Bot Mar* 54:355–365.

Spurkland T, Iken K (2012) Seasonal growth patterns of *Saccharina latissima* (Phaeophyceae, Ochrophyta) in a glacially-influenced subarctic estuary. *Phycol Res* 60:261–275.

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: Biodiversity, stability, resilience and future. *Environ Conserv* 29:436–459.

Stengel DB, Connan S, Popper ZA (2011) Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. *Biotechnol Adv* 29:483–501.

Stévant P, Rebours C, Chapman A (2017) Seaweed aquaculture in Norway: Recent industrial developments and future perspectives. *Aquac Int* 25:1373–1390.

Strain EMA, Van Belzen J, Van Dalen J, Bouma TJ, Airoldi L (2015) Management of local stressors can improve the resilience of marine canopy algae to global stressors. *PLoS One* 10:e0120837.

Straub SC, Wernberg T, Thomsen MS, Moore PJ, Burrows MT, Harvey BP, Smale DA (2019) Resistance, extinction, and everything in between – The diverse responses of seaweeds to marine heatwaves. *Front Mar Sci* 6:763.

Sunday JM, Pecl GT, Frusher S, Hobday AJ, Hill N, Holbrook NJ, Edgar GJ, Stuart-Smith R, Barrett N, Wernberg T, Watson RA, Smale DA, Fulton EA, Slawinski D, Feng M, Radford BT, Thompson PA, Bates AE (2015) Species traits and climate velocity explain geographic range shifts in an ocean-warming hotspot. *Ecol Lett* 18:944–953.

Sundfjord A, Albretsen J, Kasajima Y, Kohler J, Nuth C, Skarðhamar J, Cottier F, Nilsen F, Asplin L, Gerland S, Torsvik T (2017) Effects of glacier runoff and wind on surface layer dynamics and Atlantic water exchange in Kongsfjorden, Svalbard; a model study. *Estuar Coast Shelf Sci* 187:260–272.

Supratya VP, Coleman LJM, Martone PT (2020) Elevated temperature affects phenotypic plasticity in the bull kelp (*Nereocystis luetkeana*, Phaeophyceae). *J Phycol* 1541:1534–1541.

Svendsen H, Beszczynska-Møller A, Hagen JO, Levauconnier B, Vigdis T, Gerland S, Børre Ørbæk J, Bischof K, Papucci C, Zajaczkowski M, Azzolini R, Bruland O, Wiencke C, Winther J-G, Dallmann W (2002) The physical environment of Kongsfjorden-Krossfjorden, an Arctic fjord system in Svalbard. *Polar Res* 21:133–166.

Teagle H, Hawkins SJ, Moore PJ, Smale DA (2017) The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *J Exp Mar Bio Ecol* 492:81–98.

Terblanche JS, Deere JA, Clusella-trullas S, Janion C, Chown SL (2007) Critical thermal limits depend on methodological context. *Proc R Soc B* 274:2935–2942.

Thomas CD (2010) Climate, climate change and range boundaries. *Divers Distrib* 16:488–495.

Thomsen MS, Mondardini L, Alestra T, Gerrity S, Tait L, South PM, Lilley SA, Schiel DR (2019) Local extinction of bull kelp (*Durvillaea* spp.) due to a marine heatwave. *Front Mar Sci* 6:1–10.

tom Dieck (Bartsch) I (1992) North Pacific and North Atlantic digitate *Laminaria* species (Phaeophyta): Hybridization experiments and temperature responses. *Phycologia* 31:147–163.

tom Dieck I (1993) Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): Ecological and biogeographical implications. *Mar Ecol Prog Ser* 100:253–264.

Tuya F, Cacabelos E, Duarte P, Jacinto D, Castro JJ, Silva T, Bertocci I, Franco JN, Arenas F, Coca J, Wernberg T (2012) Patterns of landscape and assemblage structure along a latitudinal gradient in ocean climate. *Mar Ecol Prog Ser* 466:9–19.

United Nations Environment Programme (2014) Environmental effects of ozone depletion and its interactions with climate change: 2014 assessment.

Vásquez JA (2008) Production, use and fate of Chilean brown seaweeds: Re-sources for a sustainable fishery. In: *19th International Seaweed Symposium. Developments in Applied Phyc*ology, Vol 2. Borowitzka MA, Critchley AT, Kraan S, Peters A, Sjøtun K, Notoya M (eds) Springer, Dordrecht. p 7–17.

Visch W, Rad-Menéndez C, Nylund GM, Pavia H, Ryan MJ, Day J (2019) Underpinning the development of seaweed biotechnology: Cryopreservation of brown algae (*Saccharina latissima*) gametophytes. *Biopreserv Biobank* 17:378–386.

Voerman SE, Llera E, Rico JM (2013) Climate driven changes in subtidal kelp forest communities in NW Spain. *Mar Environ Res* 90:119–127.

Walczowski W, Piechura J, Goszczko I, Wieczorek P (2012) Changes in Atlantic water properties: An important factor in the European Arctic marine climate. *ICES J Mar Sci* 69:864–869.

Wernberg T, Bennett S, Babcock RC, Bettignies T De, Cure K, Depczynski M, Dufois F, Fromont J, Fulton CJ, Hovey RK, Harvey ES, Holmes TH, Kendrick GA, Radford B, Santana-garcon J, Saunders BJ, Smale DA, Thomsen MS, Tuckett CA, Tuya F (2015) Climate-driven regime shift of a temperate marine ecosystem. *Science* 353:169–172.

Wernberg T, de Bettignies T, Joy BA, Finnegan PM (2016) Physiological responses of habitat-forming seaweeds to increasing temperatures. *Limnol Oceanogr* 61:2180–2190.

Wernberg T, Filbee-Dexter K (2019) Missing the marine forest for the trees. *Mar Ecol Prog Ser* 612:209–215.

Wernberg T, Krumhansl K, Filbee-Dexter K, Pedersen MF (2019) Status and trends for the world's kelp forests. In: *World Seas: An environmental evaluation*, Second Edi. Elsevier Ltd., p 57–78.

Wernberg T, Thomsen MS, Tuya F, Kendrick GA, Staehr PA, Toohey BD (2010) Decreasing resilience of kelp beds along a latitudinal temperature gradient: Potential implications for a warmer future. *Ecol Lett* 13:685–694.

Westmeijer G, Everaert G, Pirlet H, De Clerck O, Vandegehuchte MB (2019) Mechanistic niche modelling to identify favorable growth sites of temperate macroalgae. *Algal Res* 41:101529.

White N, Marshall CE (2007) *Saccharina latissima* Sugar kelp. In: *Marine life information network: Biology and sensitivity key information reviews* [online]. Tyler-Walters H, Hiscock K (eds) Marine Biological Association of the United Kingdom, Plymouth.

Wiencke C, Bischof K (2012) Seaweed Biology – Novel insights into ecophysiology, ecology and utilization. Springer, Heidelberg New York Dodrecht London.

Wiencke C, Bischoff B, Bartsch I, Peters AF, Breeman AM (1994) Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. *Bot Mar* 37:247–260.

Wiencke C, Gómez I, Dunton K (2009) Phenology and seasonal physiological performance of polar seaweeds. *Bot Mar* 52:585–592.

Wiencke C, Roleda MY, Gruber A, Clayton M, Bischof K (2006) Susceptibility of zoospores to UV radiation determines upper depth distribution limit of Arctic kelps: Evidence through field experiments. *J Ecol* 94:455–463.

Wiencke C, Vögele B, Kovaltchouk NA, Hop H (2004) Species composition and zonation of marine benthic macroalgae at Hansneset in Kongsfjorden, Svalbard. In: *The coastal ecosystem of Kongsfjorden, Svalbard. Synopsis of biological research performed at the Koldewey Station in the years 1991 – 2003*. Wiencke C (ed) Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, p 55–62.

Wilce RT, Dunton KH (2014) The Boulder Patch (North Alaska, Beaufort Sea) and its benthic algal flora. *Arctic* 67:43–56.

Winters G, Nelle P, Fricke B, Rauch G, Reusch TBH (2011) Effects of a simulated heat wave on photophysiology and gene expression of high- and low-latitude populations of *Zostera marina*. *Mar Ecol Prog Ser* 435:83–95.

Yamaguchi T, Ikawa T, Nisizawa K (1966) Incorporation of radioactive carbon from $H^{14}CO_3^-$ into sugar constituents by a brown alga, *Eisenia bicyclis*, during photosynthesis and its fate in the dark. *Plant Cell Physiol* 7:217–229.

Zacher K, Bernard M, Bartsch I, Wiencke C (2016) Survival of early life history stages of Arctic kelps (Kongsfjorden, Svalbard) under multifactorial global change scenarios. *Polar Biol* 39:2009–2020.

Zhou B, Tang X, Wang Y (2010) Salicylic acid and heat acclimation pretreatment protects *Laminaria japonica* sporophyte (Phaeophyceae) from heat stress. *Chinese J Oceanol Limnol* 28:924–932.

Acknowledgments

First and foremost: Thank you, **Kai Bischof**, for giving me the opportunity to do my doctorate on such a great topic. Thank you for supporting all of my ideas and plans, and thank you a lot for your mentoring, guidance, encouragement and continuous support!

Special thanks go to **Ulf Karsten** for guiding me since my Bachelor's, for supporting and pushing my phycological interests and knowledge, for reviewing this thesis, and for reminding me to always rely on my instinct when it comes to big decisions.

Thank you very much to **Martin Diekmann** for agreeing to chair the commission, and also thank you to the other members of my examination commission.

I am very grateful to **Sarina Niedzwiedz** and **Niko Steiner** for being such great and helpful Bachelor students, and also to all other **co-authors** for their assistance.

A huge thank you to the very best **official and 'unofficial' Marine Botanists** for the wonderful time with many laughs, and for your tremendous support in the past 3 ½ years, even under the special conditions in the last year and over long distances – and especially to **Britta Meyer-Schlosser** for being the very best working trip buddy.

Thank you to **Inka Bartsch**, **Andreas Wagner**, **Claudia Daniel** and **Daniel Liesner** for giving me the opportunity to conduct my experiments at AWI, and for supporting me in so many ways. I really enjoyed working with you.

Thank you to **'my' working group in Rostock** for always welcoming me back as if I had never left. It was always a great pleasure! Thanks, **Juliane Müller** and **Svenja Heesch**, for your huge help in the lab.

I would like to thank the **AWI diving team** for sampling on Helgoland and Spitsbergen. Thank you **Maikee Roleda**, **Cátia Monteiro**, **Sanna Matsson**, **Sophie Steinhagen**, **Katharina Romoth**, **Myriam Valero**, **Christophe Destombe**, and **Louise Fouqueau**, for your help in collecting *Saccharina* all along the European Coast.

'A liiiiiittle' thank you to the entire **Spitsbergen Crew** for the great time in Ny-Ålesund, and that we are 'still standing'.

Ein riesiges Dankeschön geht an meine gesamte **Familie** und all die vielen **Freunde**, ohne die ich das alles (nicht nur) in den letzten Monaten niemals geschafft hätte.

Allen voran, DANKE lieber **Leo**, dass du früher Delfinforscher werden wolltest und ich eigentlich deswegen heute Meeresbiologin bin und dass du mich in so vielerlei Hinsicht unterstützt und immer für mich da bist! Danke, an **Carina** und **Aaron** für die Unterstützung und das regelmäßige Lächeln-auf-die-Lippen-Zaubern.

Besonders bedanken möchte ich mich auch bei **Elisa** für unsere Lachanfälle, deine Geduld und das Allround-Bemuttern; bei **Aurelia** und **Kurt** für eure schnellen Korrekturen, obwohl ihr so viel anderes um die Ohren habt; bei **Jessica** für die wunderschöne Gestaltung der Titelseite; bei **Malin**, **Lena** und **Laura** für diverse gelaufene Kilometer und eure immer offenen Ohren; und bei **Basti** für deine regelmäßigen Erinnerungen an Mittagessen und Feierabend und für so viel mehr!

Danke an alle (!) meine **Wormser** und „**Rostocker**“ für so viel Herzenswärme über große Distanzen und an die **Bremer**, dass ich mich all dem Wind und Regen zum Trotz hier so zuhause fühlen kann!

Und zu allerletzt, dafür aber extra groß: DANKE, liebe **Eltern**, dafür, dass ihr immer hinter mir gestanden und mich aufgefangen habt, mich bei all meinen Entscheidungen bestärkt und mir immer den Rücken freigehalten habt!!

Contact

Nora Erni Jutta Diehl, *30.11.1991 in Zweibrücken, Germany

Marine Botany
University of Bremen
Leobener Str. NW2, 28359 Bremen, Germany
ndiehl@uni-bremen.de

ORCID iD: 0000-0002-7245-340X

Publications

Diehl N, Bischof K (2021) Coping with a changing Arctic: mechanisms of acclimation in the brown seaweed *Saccharina latissima* from Spitsbergen. *Mar Ecol Prog Ser* 657:43–57.

Kuriyama K, Gründling-Pfaff S, Diehl N, Wölfel J, Karsten U (2021) Microphytobenthic primary production on exposed coastal sandy sediments of the Southern Baltic Sea using ex situ sediment cores and oxygen optodes. *Oceanologia* 63:247–260.

Monteiro C, Li H, Diehl N, Collén J, Heinrich S, Bischof K, Bartsch I (2021) Modulation of physiological performance by temperature and salinity in the sugar kelp *Saccharina latissima*. *Phycol Res* 69:48–57.

Diehl N, Karsten U, Bischof K (2020) Impacts of combined temperature and salinity stress on the endemic Arctic brown seaweed *Laminaria solidungula* J. Agardh. *Polar Biol* 43:647–656.

Liesner D, Shama LNS, Diehl N, Valentin K, Bartsch I (2020) Thermal plasticity of the kelp *Laminaria digitata* (Phaeophyceae) across life cycle stages reveals the importance of cold seasons for marine forests. *Front Mar Sci* 7:456.

Diehl N, Michalik D, Zuccarello GC, Karsten U (2019) Stress metabolite pattern in the eulittoral red alga *Pyropia plicata* (Bangiales) in New Zealand – mycosporine-like amino acids and heterosides. *J Exp Mar Bio Ecol* 510:23–30.

Scheschonk L, Becker S, Hehemann JH, Diehl N, Karsten U, Bischof K (2019) Arctic kelp eco-physiology during the polar night in the face of global warming: A crucial role for laminarin. *Mar Ecol Prog Ser* 611:59–74.

Diehl N, Kim GH, Zuccarello GC (2017) A pathogen of New Zealand *Pyropia plicata* (Bangiales, Rhodophyta), *Pythium porphyrae* (Oomycota). *Algae* 32:29–39.

Versicherung an Eides Statt

Ich, Nora Erni Jutta Diehl
28195 Bremen

versichere an Eides Statt durch meine Unterschrift, dass ich die vorstehende Arbeit selbstständig und ohne fremde Hilfe angefertigt und alle Stellen, die ich wörtlich oder dem Sinne nach aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe, mich auch keiner anderen als der angegebenen Literatur oder sonstiger Hilfsmittel bedient habe.

Ich versichere an Eides Statt, dass ich die vorgenannten Angaben nach bestem Wissen und Gewissen gemacht habe und dass die Angaben der Wahrheit entsprechen und ich nichts verschwiegen habe.

Die Strafbarkeit einer falschen eidesstattlichen Versicherung ist mir bekannt, namentlich die Strafandrohung gemäß § 156 StGB bis zu drei Jahren Freiheitsstrafe oder Geldstrafe bei vorsätzlicher Begehung der Tat bzw. gemäß § 161 Abs. 1 StGB bis zu einem Jahr Freiheitsstrafe oder Geldstrafe bei fahrlässiger Begehung.

Bremen, 20.04.2021