Thermal trait variability of the kelp *Laminaria digitata* across populations and life cycle stages

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Dissertation

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Title Figure *Laminaria digitata* in the intertidal of Helgoland, North Sea. Photo by Inka Bartsch used with permission.

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Summary

Along temperate to polar rocky shorelines, marine forests are formed by populations of large brown algae known as kelps (*sensu lato*, several orders in the family Phaeophyceae; *sensu stricto*, Laminariales). Kelps provide a three-dimensional habitat for many associated species, they bind and sequester carbon, contribute to coastal protection, extract nutrients from seawater, and are processed industrially for valuable compounds. Ocean warming is posing an increasing threat to kelps at their warm range edges and first range shifts have been recorded. For these sessile species unable to migrate, trait variability due to phenotypic plasticity and genetic variation is an important mechanism of response to environmental change. Meanwhile, evidence is accumulating for high trait variability among populations and individuals within kelp species, showing potential of marine forests to cope with ocean warming. However, these potentially interactive levels of variability have not been integrated yet for a single species. The aim of this dissertation was thus to produce a comprehensive assessment of the variation and plasticity of thermal traits across populations and life cycle stages of a keystone marine forest species, the cold-temperate to Arctic kelp *Laminaria digitata*.

To identify differentiation in heat resilience among *L. digitata* populations across the species' entire Northeast Atlantic to Arctic distribution range, I conducted a common garden heat stress experiment applying 15–23°C on sporophyte meristem tissue (**Publication I**). The upper thermal tolerance of *L. digitata* was nearly identical as material from all populations ceased growth following an exposure of 23°C for five days. However, subtle differences in growth and stress responses were revealed for three populations from the species' ecological range margins. Two populations at the species' warm distribution limit showed higher temperature tolerance compared to other populations. At 19–23°C, *L. digitata* from Quiberon (France) was most resilient in growth while material from Helgoland (North Sea) showed reduced stress responses in photosynthetic quantum yield and xanthophyll pigment accumulation. In *L. digitata* from the northernmost population of Spitsbergen (Norway), quantum yield indicated the highest heat sensitivity. Microsatellite genotyping revealed all sampled populations to be genetically distinct, with a strong hierarchical structure between southern and northern clades. The divergence of *L. digitata* into distinct clades may have facilitated phenotypic differentiation among populations at large spatial scales.

On a smaller scale, I investigated how temperature experienced during the life cycle affects phenotypic plasticity across haploid gametophyte and diploid sporophyte generations (**Publication II**). Five distinct genetic lines were initiated by separately releasing meiospores from five wild *L. digitata* sporophytes from Helgoland. Genetic lines were cultivated at the contrasting temperatures of 5 and 15°C in a full-factorial approach across three steps: (1) from meiospore germination over gametogenesis to recruitment of offspring sporophytes, (2) rearing of juvenile sporophytes and (3) a 12-day experiment assessing thermal plasticity of 3–4 month-old offspring sporophytes. This created a total of eight temperature history treatments within each genetic line. Gametogenesis and

sporophyte recruitment at 5°C promoted generally increased growth of juvenile sporophytes across experimental temperatures and genetic lines. In contrast, growth and biochemical trait performance at 5 or 15°C differed among genetic lines, partially even showing opposite response patterns, which indicates genetic variation for phenotypic plasticity. Interestingly, genetic variation for plasticity was only significant in sporophytes which had been reared at 5°C. A subsequent experiment (Master's thesis, C. Gauci) provided evidence for increased resilience of sporophytes at extreme temperatures (0 and 20°C) if they were recruited from gametophytes which had been pre-cultivated for three years at 5°C in contrast to pre-cultivation at 15°C. Both experiments provide evidence for non-genetic carry-over and cross-generational effects which modulated trait plasticity of *L. digitata* and provided benefits following cold temperature during reproduction and ontogeny.

Additionally to non-genetic effects, I investigated genetic inheritance of thermal traits by making use of the genetic and physiological differentiation among Helgoland and Spitsbergen *L. digitata* (**Publication III**). Sporophyte lineages produced by in- and outbreeding of gametophyte isolates from the two populations were subjected to sublethal and lethal temperatures in two common garden experiments. In the first experiment on microscopic sporophytes, the upper survival temperature over 14 days was lower for the inbred Spitsbergen selfing (21°C) than for the Helgoland selfing and the outbred reciprocal crosses (22°C), which indicates mid-parent heterosis in the crosses. In the second experiment, I subjected macroscopic sporophytes to a control temperature (10°C), moderate (19°C) and critical heat stress (20.5°C) to assess metabolic regulation via whole-transcriptome analysis in addition to physiological parameters. The Spitsbergen selfing died within seven days in the heat treatments and showed the highest differential gene expression among the lineages at 10°C. Considering only the three surviving lineages at 20.5°C, differential gene expression was reduced in the reciprocal crosses compared to the Helgoland selfing, despite their similar physiological responses in growth and optimum quantum yield. Among the identified transcripts, gene expression related to cellular stress responses was reduced in the reciprocal crosses compared to the Helgoland selfing at 20.5°C. These results imply that thermal traits are inherited from both female and male gametophyte parents. In addition, the intraspecific crosses maintained a similar physiology to the inbred Helgoland selfing with reduced metabolic regulation during sublethal heat stress, which may be a beneficial effect of outbreeding even with a less heat-tolerant lineage.

This dissertation therefore presents evidence for four levels of thermal trait variability in a marine forest key species. In the publications presented here, I describe **(1)** genetic and physiological differentiation among populations, **(2)** genetic variation for phenotypic plasticity among genotypes, **(3)** carry-over and cross-generational effects over reproduction and individual ontogeny, and **(4)** the production of new phenotypes by outbreeding among distant lineages. Integrating these responses into a framework of seasonal temperature variation and predictions of ocean warming showed that *L. digitata*, as a cold-temperate to Arctic species, is adapted well to the current conditions along its

distributional range, but may not be equipped to respond to rapid climate change at its warm range edges. The concepts investigated in this dissertation provide further insight into trait variability as a mechanism of marine forest resilience, but they also describe intriguing features for mariculture and conservation efforts. Continuing research on genetic variation for plasticity, heterosis, and cross-generational plasticity in kelps may therefore provide powerful tools to produce productive marine crops and to restore natural marine forests.

Zusammenfassung

Entlang gemäßigter bis polarer Felsküsten bilden große Braunalgen (Kelp; *sensu lato*, mehrere Ordnungen in der Familie Phaeophyceae; *sensu stricto*, Laminariales) die Grundlage der Kelpwälder. Diese artenreichen Ökosysteme binden Kohlendioxid, tragen zum Küstenschutz bei, entziehen dem Seewasser Nährstoffe, und werden industriell zur Produktion hochwertiger Inhaltsstoffe verwendet. Durch Ozeanerwärmung sind Kelps zunehmend an ihren warmen Verbreitungsgrenzen bedroht und erste Artenverschiebungen wurden bereits beobachtet. Die festsitzenden Kelps sind angewiesen auf Merkmalsvariation durch phänotypische Plastizität und genetische Varianz um sich an eine sich verändernde Umwelt anzupassen. Mittlerweile häufen sich Beweise für starke Merkmalsvariation innerhalb von Kelp-Arten zwischen Populationen und zwischen Individuen, was darauf hinweist, dass sich Kelpwälder an die Ozeanerwärmung anpassen könnten. Diese wahrscheinlich interaktiven Ebenen der Variation wurden allerdings bisher nicht für eine Art übergreifend untersucht. Das Ziel dieser Dissertation war es daher, die Varianz und Plastizität temperaturabhängiger Merkmale einer Schlüsselart der Kelpwälder, des kalt-gemäßigten bis Arktischen Fingertangs *Laminaria digitata*, über Populationen und Lebenszyklusstadien hinweg zu untersuchen.

Um Unterschiede in der Hitzetoleranz zwischen *L. digitata* Populationen entlang der gesamten Verbreitung der Art vom Nordostatlantik bis in die Arktis zu ermitteln, habe ich ein Laborexperiment durchgeführt, in dem ich Meristemscheiben denselben Temperaturen von 15–23°C ausgesetzt habe (**Publikation I**). Die obere Temperaturtoleranz war nahezu identisch, da Proben von allen Standorten nach fünf Tagen bei 23°C das Wachstum einstellten. Allerdings traten feine Unterschiede in Wachstum und Stresssymptomen in drei Populationen an den ökologischen Verbreitungsgrenzen hervor. Zwei Populationen an der warmen Verbreitungsgrenze zeigten eine erhöhte Hitzetoleranz verglichen mit den anderen Populationen. Bei 19–23°C war das Wachstum von *L. digitata* aus Quiberon (Frankreich) widerstandsfähiger, während Material aus Helgoland (Nordsee) verringerte Stressantworten in photosynthetischer Quantenausbeute zeigte und weniger Xanthophyllpigmente anreicherte. Die Quantenausbeute von *L. digitata* aus der nördlichsten Population (Spitzbergen, Norwegen) zeigte die höchste Empfindlichkeit für Hitze. Genotypisierung mittels Mikrosatelliten zeigte, dass alle Populationen genetisch getrennt waren, während eine hierarchische Struktur je eine übergeordnete südliche und nördliche Gruppe eingrenzte. Das legt nahe, dass sich *L. digitata* über Eiszeitalter hinweg in zwei Gruppen gespalten hat, was wiederum die phänotypische Differenzierung zwischen Populationen auf einer großen räumlichen Skala begünstigt haben kann.

Auf einem kleineren Maßstab habe ich untersucht, wie der Temperaturverlauf während des Lebenszyklus die phänotypische Plastizität zwischen den Generationen haploider Gametophyten und diploider Sporophyten beeinflusst (**Publikation II**). Basierend auf Meiosporen von fünf wilden *L. digitata* Sporophyten von Helgoland wurden fünf getrennte genetische Linien erzeugt. Die genetischen Linien wurde bei den gegensätzlichen Temperaturen 5 und 15°C in einem

vollfaktoriellen Ansatz über drei Stufen kultiviert: (1) von der Sporenkeimung über die Gametogenese bis zur Rekrutierung junger Sporophyten-Nachkommen, (2) über die Aufzucht junger Sporophyten (3) zu einem 12-tägigen Experiment zur Temperatur-Plastizität von 3–4 monatealten Sporophyten. Dadurch entstanden acht verschiedene Temperaturhintergründe in jeder genetischen Linie. Gametogenese und Rekrutierung bei 5°C führte zu generell besserem Wachstum der Sporophyten-Nachkommen im Experiment. Zusätzlich variierte die Plastizität von Wachstum und biochemischen Parametern bei 5 und 15°C zwischen den genetischen Linien, teilweise mit entgegengesetzten Reaktionsmustern, was auf genetische Variation für Plastizität hinweist. Interessanterweise war dieser Effekt nur signifikant bei Sporophyten, die bei 5°C und nicht bei 15°C aufgezogen wurden. Ein anschließendes Experiment (Masterarbeit, C. Gauci) lieferte Beweise für gesteigerte Widerstandsfähigkeit von Sporophyten bei extremen Temperaturen (0 und 20°C), wenn sie von Gametophyten abstammen, die für drei Jahre bei 5°C, im Gegensatz zu 15°C, kultiviert wurden. Diese Ergebnisse liefern Beweise für nicht-genetische carry-over und transgenerationelle Effekte, die entlang der individuellen Entwicklung und über Generationen hinweg die Merkmalsplastizität von *L. digitata* beeinflussen, und zeigen Vorteile kalter Temperaturen während der Reproduktion und Ontogenese von *L. digitata* auf.

Zusätzlich zu nicht-genetischen Effekten habe ich die genetische Vererbung temperaturabhängiger Merkmale untersucht, indem ich die genetische und physiologische Differenzierung zwischen *L. digitata* von Helgoland und Spitzbergen genutzt habe (**Publikation III**). Sporophyten verschiedener Abstammungslinien wurden durch Inzucht und Kreuzung zwischen Gametophyten-Isolaten aus beiden Populationen erzeugt und in zwei Experimenten auf ihre Reaktion auf subletale und letale Temperaturen untersucht. Im ersten Experiment an mikroskopischen Sporophyten war die obere Überlebenstemperatur über 14 Tage geringer in der Spitzbergen Inzuchtlinie (21°C) als in der Helgoland Inzuchtlinie und den reziproken Kreuzungen (22°C), was auf Heterosis in den Kreuzungen hinweist. Im zweiten Experiment habe ich makroskopische Sporophyten bei einer Kontrolltemperatur (10°C) und unter moderatem (19°C) und kritischem Hitzestress (20.5°C) auf physiologische Reaktion und metabolische Regulation mittels Transkriptomanalyse untersucht. Sporophyten der Spitzbergen Inzuchtlinie waren in den Hitzebehandlungen innerhalb von sieben Tagen abgestorben und zeigten die höchste Genregulation unter den Abstammungslinien bei 10°C. In einem Vergleich der drei überlebenden Abstammungslinien bei 20.5°C war die differentielle Genregulation in den reziproken Kreuzungen geringer als in der Helgoland Inzuchtlinie, obwohl alle Linien in Wachstum und photosynthetischer Quantenausbeute ähnlich reagierten. Verglichen mit der Helgoland Inzuchtlinie war die Expression von Genen für zelluläre Stressreaktionen in den reziproken Kreuzungen bei 20.5°C reduziert. Diese Ergebnisse zeigen, dass Temperaturmerkmale von weiblichen sowie von männlichen Gametophyten vererbt werden. Zusätzlich haben die innerartlichen Kreuzungen unter subletalem Hitzestress mit reduzierter Genregulation eine ähnliche

Physiologie aufrechterhalten wie die Helgoland Inzuchtlinie, was ein positiver Effekt der Kreuzung mit einer anderen, sogar einer weniger hitzetoleranten, Abstammungslinie sein kann.

Dementsprechend liefert diese Dissertation Beweise für vier Ebenen temperaturabhängiger Merkmalsvariation in einer Schlüsselart mariner Kelpwälder. In den Publikationen, die dieser Dissertation zugrunde liegen, beschreibe ich **(1)** genetische und physiologische Differenzierung zwischen Populationen, **(2)** genetische Variation für phänotypische Plastizität zwischen Genotypen, **(3)** nicht-genetische Effekte entlang der individuellen Entwicklung und über Generationen hinweg, und **(4)** die Bildung neuer Phänotypen durch Kreuzung entfernter Abstammungslinien. Die Integration dieser Merkmale im Kontext saisonaler Temperaturschwankungen und Vorhersagen der Ozeanerwärmung zeigt, dass *L. digitata* als kalt-gemäßigte bis arktische Art gut an die vorherrschenden Bedingungen entlang ihrer Verbreitung angepasst ist. Allerdings ist sie möglicherweise nicht ausgerüstet, sich dem schnellen Klimawandel an ihrer warmen Verbreitungsgrenze anzupassen. Die Konzepte, die in dieser Dissertation untersucht wurden, liefern Erkenntnisse über die Funktion von Merkmalsvariation für die Widerstandsfähigkeit mariner Kelpwälder, aber sie sind auch von Bedeutung für Marikultur und Erhaltungsmaßnahmen. Weiterführende Untersuchungen zu genetischer Variation für Plastizität, Heterosis und transgenerationelle Effekte in Kelp-Arten können daher wirksame Werkzeuge entwickeln, um produktive marine Algenkultivare zu züchten und um natürliche Kelpwälder zu erhalten.

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Abbreviations

α	Photosynthetic efficiency
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
bp	Base pair
C	Carbon
C_i	Inorganic carbon
C:N ratio	Carbon : nitrogen ratio
CA	Carbonic anhydrase
CCM	Carbon concentrating mechanism
CGP	Cross-generational plasticity
Chl *a*	Chlorophyll *a*
CO_2	Carbon dioxide
COE	Carry-over effect
CSR	Cellular stress response
CTAB	Cetyltrimethylammonium bromide
DEG	Differentially expressed gene
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DW	Dry weight
E_{50}	Saturation irradiance of nonphotochemical quenching
ETR	Electron transport rate
F_v/F_m	Optimum quantum yield
GxE	Genotype x environment interaction
HCO_3^-	Bicarbonate
HPLC	High-performance liquid chromatography
HSP	Heat shock protein
I_k	Saturation irradiance of photosynthesis
Log_2FC	Log_2-fold change
LHC	Light harvesting complex
MGDG	Monogalactosyldiacylglycerol
MPH	Mid-parent heterosis
N	Nitrogen
n (NPQ)	Sigmoidicity coefficient of nonphotochemical quenching

n (Statistics)	Number of replicates per treatment
NPQ	Nonphotochemical quenching
NPQ_{max}	Maximum nonphotochemical quenching
PAM	Pulse-amplitude modulation
PAR	Photosynthetically active radiation
PCA	Principal component analysis
PES	Provasoli enriched seawater
PFD	Photon flux density
pH	Negative decimal logarithm of the hydronium ion activity in a solution
POC	Particulate organic carbon
PS II	Photosystem II
PUFA	Polyunsaturated fatty acid
QTL	Quantitative trait locus
RCP	Representative concentration pathway of atmospheric greenhouse gases
$rETR_{max}$	Maximum relative electron transport rate
RLC	Rapid light curve
RM ANOVA	Repeated measures analysis of variance
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPKM	Reads per kilobase of transcript per million mapped reads
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
SD	Standard deviation
SE	Standard error
SNP	Single nucleotide polymorphism
SST	Sea surface temperature
SW	Seawater
UV	Ultraviolet (radiation)
VAZ	Xanthophyll pigment pool (violaxanthin + antheraxanthin + zeaxanthin)
VDE	Violaxanthin de-epoxidase

Glossary

Acclimation is the fast and reversible physiological adjustment of an individual to enhance or maintain performance in response to an environment and is an expression of *phenotypic plasticity* (Leroi et al., 1994; Reusch, 2014).

Adaptation occurs over generations of selection on heritable traits of *phenotypes* (Morgan-Kiss et al., 2006; Donelson et al., 2019) and may alter a population's ecological niche towards a local optimum (King et al., 2018).

Carry-over effect (COE) = Developmental plasticity describes *phenotypic* changes which occur within the development of an individual and may alter trait expression at a later time point; e.g. adjusted morphology in adults due to stress during ontogeny (Palmer et al., 2012; Byrne et al., 2020).

Cross-generational plasticity (CGP) describes the interaction of parental (F0 generation) environment and offspring (F1 generation) environment in shaping the offspring's *phenotypic plasticity* (i.e. the shape of the *reaction norm*; Byrne et al., 2020).

Ecotype describes a variant of a species, which is the result of the *genotypical* response of a species to selection by a particular habitat; e.g. locally *adapted* populations (Turesson, 1922; Gregor, 1944).

Fitness is a measure of an individual's ability to survive and produce viable offspring in the prevailing environment (Darwin, 1859; Orr, 2009).

Genetic drift is a change in allele frequencies by chance due to random reproduction (Bolnick et al., 2011). It is especially strong in small populations following e.g. bottleneck events.

Genetic variation for plasticity describes differences in *phenotypic plasticity* among *genotypes*, in which the extent of trait plasticity itself is an *adaptive* trait on which natural selection can act (Newman, 1994). Genetic variation for plasticity can be identified as significant *genotype x environment interactions*.

Genotype in a population genetic sense describes the entire genomic sequence of an organism, while *sensu lato*, the term can address individuals with nearly identical genomes such as inbred strains or clones (Nicotra et al., 2010).

Genotype x environment interaction (GxE) is a measure of differentiation among individuals' *phenotypic plasticity* (Via and Lande, 1985; Saltz et al., 2018). It is visualised as differently shaped *reaction norms* among *genotypes* and can be used to identify *genetic variation for plasticity*.

Germline defines the reproductive lineage of cells in an organism starting with the first cell which is committed to produce gametes (Grossniklaus, 2011; Schmidt et al., 2015). In kelps, this definition encompasses all cells in the life cycle from the meiosporangium mother cell formed by the sporophyte in soral tissue to the oogonia and spermatia produced by female and male gametophytes.

Heterosis = Hybrid vigour is the superior performance of any trait (e.g. growth, developmental speed and/or fertility) in inter- and intraspecific crosses in comparison to their genetically different inbred parents (Hochholdinger and Hoecker, 2007; Birchler et al., 2010). In mid-parent heterosis, the hybrid performs significantly better than the average performance over both parental lineages, whereas in best-parent heterosis, hybrid performance surpasses the better of the two parents (Hochholdinger and Hoecker, 2007).

Inbreeding is the mating of two genetically similar, closely related genotypes (Charlesworth and Charlesworth, 1987) and may lead to *inbreeding depression* in the resulting offspring. The most extreme form of inbreeding is *selfing*.

Inbreeding depression is reduction of performance in offspring of closely related individuals which is attributed to the accumulation of harmful recessive traits or to general disadvantages of homozygosity (Charlesworth and Charlesworth, 1987; Schierup and Christiansen, 1996).

Maladaptation occurs in populations which deviate in performance from an *adaptive* optimum, e.g. due to mutation or a lack of *genotypic* and *phenotypic* variation to respond to natural selection (Crespi, 2000; Brady et al., 2019).

Marginal population is a population at the ecological range margin of the species (Soulé, 1973), which often, but not necessarily, is the case at the distributional range edges.

Marine heatwave is defined as an anomalously warm event lasting five or more days with seawater temperatures warmer than the 90^{th} percentile based on a 30-year historical baseline period (Hobday et al., 2016).

Outbreeding = outcrossing is the mating of two genetically differentiated (unrelated) genotypes within a species (Waser and Price, 1989), which may lead to *heterosis* or *outbreeding depression* in the resulting offspring.

Outbreeding depression is a reduction of performance in offspring of genetically differentiated lineages which is attributed to a disruption of local *adaptation* to different environments among the parental lineages or to a disruption of allele interactions (McKay et al., 2005).

Parental effect describes an influence of the parental environment on offspring trait expression regardless of offspring environment. Parental effects do therefore not alter offspring *phenotypic plasticity*, i.e. the shape of the reaction norm does not change across parent and offspring generations, but offspring trait expression may be generally higher or lower depending on the parental environment (Salinas et al., 2013).

Phenology is the timing of annually recurring life cycle events, often investigated with respect to seasonal variations in abiotic conditions (Stucky et al., 2018).

Phenotype is the appearance or characteristics of one or several traits in an individual organism resulting from both genetic and environmental influences (Nicotra et al., 2010).

Phenotypic buffering is the maintenance of the same *phenotype* across a range of environmental stressors to maintain function and tolerance (Reusch, 2014). Contrary to *phenotypic plasticity*, the reaction norm of a trait maintained by phenotypic buffering is flat for highly tolerant *genotypes*.

Phenotypic plasticity = Within-generation plasticity describes the range of *phenotypes* a single *genotype* can express as a function of its environment (Nicotra et al., 2010), e.g. highly plastic individuals can present a wide range of phenotypic responses in response to an environmental gradient.

Reaction norm describes the expected *phenotype* of a given *genotype* as a function of the environment (Chevin et al., 2010) and is often visualised as a graph depicting phenotypic trait expression over an environmental gradient. In scientific studies, reaction norms are derived from experiments with e.g. inbred lineages (Li et al., 2018), siblings (Shama, 2017) or age cohorts (Hurst et al., 2012).

Selfing is the most extreme form of *inbreeding* and describes the production of offspring from gametes of the same organism. For kelps, the term is used to describe kelp sporophytes obtained by fertilizing sibling female and male gametophytes (Raimondi et al., 2004).

"Silver spoon" parental effect is a beneficial parental effect, in which parents in favourable environments produce offspring with high levels of *fitness*-related traits, e.g. by maternal provisioning of resources to early life stages (Uller et al., 2013; Baker et al., 2019).

1 General introduction

1.1 Marine forests

Marine forests are ecosystems dominated by large seaweeds, which occur along warm-temperate to polar rocky coastlines (Lüning, 1990; Wernberg et al., 2019). Foundation species of these marine forests are brown algae of the order Phaeophyceae, which are commonly called kelp. In a functional sense, the term kelp can describe macroalgae from several brown algal orders, mainly Laminariales, Fucales, Desmarestiales and Tilopteridales (Fraser, 2012; Smale, 2020). In the strict sense, kelps are defined as large brown algae exclusively within the order Laminariales (Steneck et al., 2002; Bartsch et al., 2008). Common to either group defined as kelp is their function as ecosystem engineers, which provide three-dimensional structure above the seafloor (**Figure 1.1**) and alter the physico-chemical features of the environment (Christie et al., 2009; Fraser, 2012; Teagle et al., 2017; Pfister et al., 2019; Wernberg et al., 2019). This biogenic structure harbours a high diversity of associated organisms. For instance, canopy-forming kelp species reduce the irradiance regime and provide a habitat for undergrowth algae adapted to low irradiance (Wernberg et al., 2005; Pedersen et al., 2014), while kelps themselves are substrate for endo- and epiphytic organisms (Bartsch et al., 2008; Smale et al., 2015). Invertebrates such as molluscs, crustaceans and echinoderms inhabit and/or feed on kelp (Norderhaug et al., 2003; Christie et al., 2007, 2009; Teagle et al., 2017), and also fish may use kelp forests as nurseries and shelter (Bodkin, 1988). Dense kelp stands even enhance the diversity of the microbial community in the surrounding seawater (Pfister et al., 2019). This structure and diversity evokes the comparison to terrestrial forests, which earned marine forests their name (Wernberg and Filbee-Dexter, 2019).

Figure 1.1 Marine forest at the coast of Stavanger, Norway. The three-dimensional structure is provided by *Laminaria hyperborea*, whose stipes are colonized by an assemblage of epibionts. Photo by Uli Kunz used with permission.

Marine forests are among the most productive ecosystems in the world (Mann, 1973; Filbee-Dexter, 2020). Primary production by kelps is estimated to be of comparable range to temperate and tropical forests (Lüning, 1990; Filbee-Dexter, 2020). However, standing stock of kelps is low when compared to terrestrial and mangrove forests (Pessarrodona et al., 2018; Filbee-Dexter, 2020), as more than 80% of the assimilated carbon is released as macroscopic detritus, particulate (POC) or dissolved (DOC) organic carbon (Krumhansl and Scheibling, 2012; Pessarrodona et al., 2018; Pedersen et al., 2020). Released organic carbon either enters the food web (Lin et al., 2018; Paar et al., 2019b) or it is buried in sediment and sequestered (Krause-Jensen and Duarte, 2016; Filbee-Dexter and Wernberg, 2020). Of the estimated global net primary production of 1521 million tons C $year^{-1}$ by macroalgae, more than 10% are estimated to be sequestered (Krause-Jensen and Duarte, 2016). Therefore, macroalgae have shifted into focus of blue carbon assessments, which describe carbon capture and storage by marine organisms (Nellemann et al., 2009; Krause-Jensen et al., 2018; Filbee-Dexter and Wernberg, 2020). This sparks incentives to mitigate anthropogenic CO_2 accumulation via macroalgae (Chung et al., 2011; Duarte et al., 2017; Sondak et al., 2017; Froehlich et al., 2019).

Humans have, however, benefitted from marine forests for ages. The kelp highway hypothesis proposes that, additionally to the deglaciation of the North Pacific opening a dispersal corridor, rich marine coastal habitats may have facilitated the migration of humans from Asia into the Americas about 13,500 years ago (Erlandson et al., 2007; Braje et al., 2017). Historical use of seaweeds as food is, albeit scarce, documented all over the world (Mac Monagail et al., 2017). In Europe, kelp was used from the 17th century predominantly to produce alkali which was necessary in soap and glass production (Clow and Clow, 1947; Forsythe, 2006). Current global estimates value goods and services provided by kelp forests between 500,000 and 1,000,000 US dollars per kilometre of coastline per year (Filbee-Dexter and Wernberg, 2018). Kelps are of direct commercial value mainly because of their high concentration in valuable compounds such as pigments, lipids, proteins, phenolics and polysaccharides (Stengel et al., 2011). The polysaccharide alginate is industrially produced from kelp and fucoid seaweeds (Vásquez, 2008; Blamey and Bolton, 2018; Peteiro, 2018) and is used mainly in cosmetics, pharmaceuticals and food industry as a stabilizer and gelling agent (Peteiro, 2018). Worldwide, the majority of kelp used for alginate production is harvested from wild populations or collected from washed ashore biomass (Bixler and Porse, 2011; Rebours et al., 2014; Peteiro, 2018). In contrast, the production of kelp in the Asian food industry is centred around mariculture of the kelps *Saccharina japonica* and *Undaria pinnatifida* (Buschmann et al., 2017). Aside from the direct economic value of kelp, marine forests attract tourism and provide fruitful fishing grounds (Paddack and Estes, 2000; Blamey and Bolton, 2018). Further indirect value is gained from kelp forests' ability to remove nutrients from seawater and estuaries (Kim et al., 2015) and to mitigate coastal erosion (Blamey and Bolton, 2018; Morris et al., 2019). Therefore, conservation of kelp forests is not only of ecological importance but also of utmost economic interest.

1.2 Current threats to marine forests

Marine forests are facing substantial environmental changes. The most conspicuous and well-investigated factor of global change acting on marine systems is ocean warming (Harley et al., 2006). Changes in temperature can drive major ecological shifts, as temperature affects chemical reaction rates and physiological processes such as growth, reproduction and survival in individuals, thereby ultimately delimiting species' distribution ranges (Jeffree and Jeffree, 1994; Pörtner and Farrell, 2008; Stuart-Smith et al., 2017). On a global scale, the biogeographical distribution of seaweeds can roughly be explained by their thermal limits of reproduction, growth and survival with respect to minimum and maximum mean monthly sea surface isotherms (van den Hoek, 1982a, 1982b; Breeman, 1990; Lüning, 1990). As the oceans are warming, these isotherms have been shifting poleward for at least 60 years (Burrows et al., 2011) which affects the timing and magnitude of seasonal temperature variation (Lima and Wethey, 2012). Accordingly, many marine species have responded with distributional and phenological shifts (Poloczanska et al., 2013). Several kelp and fucoid seaweeds have already undergone poleward range shifts which were attributed to global warming (Lima et al., 2007; Nicastro et al., 2013; Smale et al., 2015) and further shifts are predicted for many seaweeds (Bartsch et al., 2012; Raybaud et al., 2013; Assis et al., 2018). In addition to the gradual increase of average ocean temperature, short, extreme warming events (marine heatwaves *sensu* Hobday et al., 2016) have been increasing in frequency and duration (Oliver et al., 2018). Marine heatwaves act as strong selective events, can alter species composition and reduce the genetic diversity of persistent populations (Arafeh-Dalmau et al., 2019; Coleman et al., 2020a; Gurgel et al., 2020). The occurrence of marine heatwaves especially during summer has recently been correlated to systemic ecosystem collapse of marine forests at their warm distributional range edges (Wernberg et al., 2016; Thomsen et al., 2019; Filbee-Dexter et al., 2020).

In contrast, ocean acidification may be buffered for kelps and associated organisms through the photosynthetic drawdown of CO_2 during the day in marine forests (Roleda and Hurd, 2012; Hurd, 2015; Ling et al., 2020). For kelps, an increased availability of dissolved inorganic carbon may ameliorate negative effects of reduced pH (Roleda et al., 2012; Leal et al., 2017). However, responses of kelps to ocean acidification may differ among habitats, species and life cycle stages (Roleda and Hurd, 2012; Britton et al., 2016; Gordillo et al., 2016). Other abiotic changes affect marine forests especially at high latitudes. For instance, increased UV radiation especially in the polar regions may damage kelp and their propagules (Karsten et al., 2009; Roleda et al., 2010; Huovinen and Gómez, 2013). Glacial melting may provide new rocky substrates for kelps to populate (Krause-Jensen and Duarte, 2014; Deregibus et al., 2016; Filbee-Dexter et al., 2019), but the runoff of glacial meltwater also drives a reduction in seawater salinity, which may pose a stressor for kelps (Fredersdorf et al., 2009; Li et al., 2019; Monteiro et al., 2019b). The sediment load in the meltwater runoff increases turbidity (i.e. coastal darkening; Aksnes et al., 2009), which may provide protection from UV

radiation (Roleda et al., 2008), but in turn narrows the depth distribution of seaweeds due to the attenuated light (Bartsch et al., 2016). Increased sedimentation may again reduce the availability of rocky substrate (Wiencke and Amsler, 2012; Zacher et al., 2016; Filbee-Dexter et al., 2019).

Abiotic stressors don't affect single organisms alone, but also modulate species interactions and can have cascading effects throughout the food web (Paar et al., 2019a; Vergés et al., 2019). For instance, marine heatwaves, ocean warming and other stressors may drive over-grazing of kelp by sea urchins, which creates stable "urchin barrens" (Johnson et al., 2011; Filbee-Dexter and Scheibling, 2014; Rogers-Bennett and Catton, 2019). In a reverse effect, an increase in the frequency of kelp-removing storms may reduce the diversity of higher trophic levels in the marine forest food web (Byrnes et al., 2011). Over the last decade, a replacement of marine forests by low-lying mats of turf algae has been observed, which drives a drastic reduction in structural complexity and food web diversity (Filbee-Dexter and Wernberg, 2018; Vergés et al., 2019). Suspected drivers of these shifts are ocean warming, heatwaves, eutrophication, and grazing pressure (Wernberg et al., 2016; Filbee-Dexter and Wernberg, 2018; Christie et al., 2019a; Straub et al., 2019). Turf ecosystems may be self-stabilizing, preventing a shift back to marine forest ecosystems by trapping sediment and attracting associated herbivores which prevent macroalgae recruitment (Vergés et al., 2014; Filbee-Dexter and Wernberg, 2018; Feehan et al., 2019). This mechanism is especially severe at the warm range edges, where the occurrence of prolonged cool conditions that would allow a recovery of marine forests is becoming increasingly unlikely (Wernberg et al., 2016; Filbee-Dexter and Wernberg, 2018). Shifts to turf ecosystems also occur in cooler regions (e.g. Norway; Moy and Christie, 2012), but there, marine forests are partially able to recover or the system may even fluctuate between the two states (Ebeling et al., 1985; Christie et al., 2019a).

Mean global land and ocean surface temperature during the year 2019 was among the three warmest annual temperatures on record (Blunden and Arndt, 2020). In the current year 2020, air temperatures reached an unprecedented 21.7°C on the archipelago of Svalbard (Nikel, 2020) and 38°C in the Siberian Arctic (Ciavarella et al., 2020). At the time of writing in September 2020, Arctic sea ice extent is the second lowest since records began (Grosfeld et al., 2016). Meanwhile, drought and high temperature fuelled extensive fires in Siberia (Stone, 2020), Australia (BBC News, 2020) and North America (Voiland, 2020) in 2019 and 2020. This year also saw the third and most extensive coral bleaching event in the Great Barrier Reef within five years (ARC Centre of Excellence for Coral Reef Studies, 2020). Despite these drastic examples and consequences of global warming, the global extent of kelp forests has only slightly declined over the past half-century (Krumhansl et al., 2016). However, trends in kelp abundance varied strongly around the world, with regions of increasing (27%), decreasing (38%), or stable (35%) kelp forest extent (Krumhansl et al., 2016). This highlights the importance of diversified research taking into account local conditions and response variation when aiming to characterize the future trajectory of marine forests during climate change.

1.3 Case species *Laminaria digitata*

1.3.1 Kelps of the North Atlantic

Laminarian kelps (kelps *sensu stricto*) have diversified between 21–43 million years ago in the North Pacific (Rothman et al., 2017; Starko et al., 2019) and are currently distributed worldwide from warm-temperate to polar latitudes along ca. 25% of the world's coastlines (Lüning, 1990; Filbee-Dexter and Wernberg, 2018; Smale, 2020). The order Laminariales (excluding the Chordales *sensu* Starko et al., 2019) contains 105 species in 30 genera (Bolton, 2010). Only few kelp genera occur in the North Atlantic, likely because of the relatively recent and challenging migration from the Pacific to the Atlantic via the Bering Strait ca. 5.3 million years ago (Lüning and tom Dieck, 1990; Bolton, 2010; Rothman et al., 2017). In the Northeast Atlantic – the research area of this study – the three kelp genera *Alaria*, *Saccharina* and *Laminaria* contain keystone species of marine forests (Bolton, 2010). When including the Arctic, the recent reassignment of *Laminaria nigripes* J.Agardh to *Hedophyllum nigripes* (J.Agardh) Starko, S.C.Lindstrom & Martone (Starko et al., 2019) adds a fourth genus to the list (Dankworth et al., 2020). Within the genus *Laminaria*, five species occur in the North Atlantic across marine biogeographical regions (*sensu* Lüning, 1990): Two deep-water kelps are endemic in contrasting regions of the Atlantic – *Laminaria solidungula* J.Agardh in the Arctic (Roleda, 2016) and *Laminaria rodriguezii* Bornet in the Mediterranean (Boisset et al., 2016). The dominating kelp in the Northeast Atlantic is *Laminaria hyperborea* (Gunnerus) Foslie, which forms dense stands in the sublittoral of cold-temperate regions between mid-Portugal and northern Norway (Kain, 1979; Araújo et al., 2016). *Laminaria ochroleuca* Bachelot de la Pylaie is a warm-temperate species occurring between Morocco and South England, from where it is currently extending northward (Smale et al., 2015). Finally, *Laminaria digitata* (Hudson) J.V.Lamouroux is a cold-temperate to Arctic species with an amphi-Atlantic distribution. In the Northeast Atlantic, it shares most of its latitudinal distribution range with *L. hyperborea* in the shallow sublittoral and infralittoral fringe. *L. digitata* occurs from the Arctic archipelago of Spitsbergen, Norway (79°N) to Quiberon in South Brittany, France (47°N) and to Long Island Sound (41°N) in the West Atlantic (Lüning, 1990; Stewart Van Patten and Yarish, 2009; Oppliger et al., 2014; Bartsch et al., 2016; Guiry and Guiry, 2020). On wave-exposed rocky coasts, it mostly extends from the infralittoral fringe to a subtidal depth of ~1.5 m (Kain, 1975; Lüning, 1990), below which it is outcompeted by *L. hyperborea* (Kain, 1975; Hawkins and Harkin, 1985). In the Arctic, where presence of *L. hyperborea* has not been confirmed (Bartsch et al., 2016; Hop et al., 2016; Fredriksen et al., 2019), *L. digitata* may occur down to 15 m depth, where it is presumably delimited by low irradiance (Bartsch et al., 2016). In the upper sublittoral, *L. digitata* is subject to strong environmental gradients and stressors. Therefore, the species has to be tolerant to desiccation stress and warm air temperature (King et al., 2018; Hereward et al., 2020), high irradiance and UV radiation (Roleda et al., 2006; Gruber et al., 2011), low salinity due to freshwater input (Karsten, 2007) and mechanical stress from wave action

(Lüning, 1990; Harder et al., 2006). These characteristics, its ecological importance as a marine forest key species and not least its relatively easy accessibility during lowest tide make *L. digitata* a compelling study object.

1.3.2 The kelp life cycle

Like all laminarian kelps, *L. digitata* alternates between microscopic, haploid gametophytes and macroscopic, diploid sporophytes in a haplo-diplontic life cycle (Bold and Wynne, 1985; van den Hoek et al., 1995). The diploid, perennial sporophytes are complex thalli, which are structured into three main organs (**Figure 1.2A**). A branched holdfast (rhizoid) attaches the kelp to a hard substrate. A flexible stipe (cauloid) allows resilience against wave action (Lüning, 1990) and the blade (lamina) provides a large surface area for photosynthesis and reproduction. At the intersection of stipe and blade lies the meristem, the zone of active intercalary growth. In contrast, gametophytes are microthalli with a much simpler structure than sporophytes (**Figure 1.2B**). They grow filamentously on benthic substrate (Robuchon et al., 2014a) or endophytically (Bringloe et al., 2018). Other than vegetative and reproductive cells, gametophytes do not develop differentiated structures.

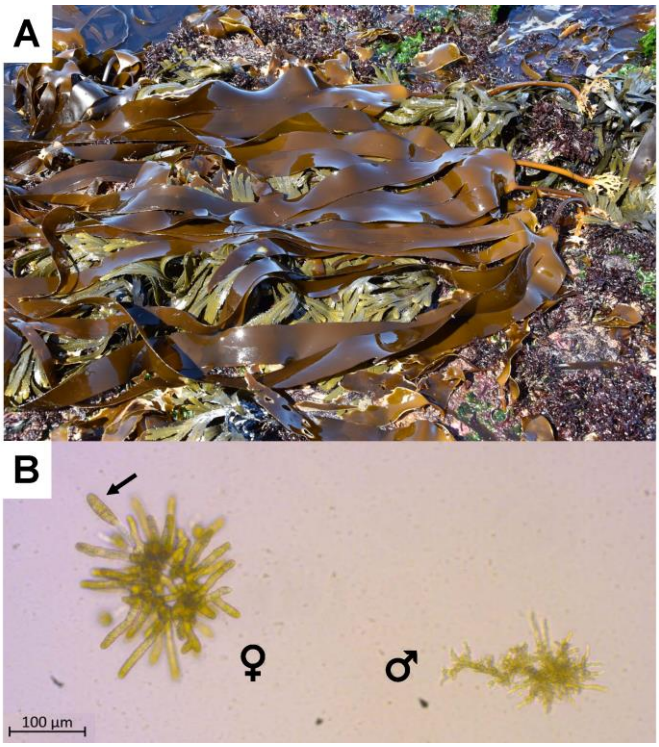

Figure 1.2 Life cycle stages of *Laminaria digitata*. **(A)** *L. digitata* sporophytes among other seaweeds on the island of Helgoland, North Sea. Photo by Andreas Wagner used with permission. **(B)** Female (♀) and male (♂) *L. digitata* gametophytes and a two-celled sporophyte (arrow). Photo by Daniel Liesner.

When sporophytes become fertile (**Figure 1.3**), parts of the somatic blade tissue differentiate into clearly segregated sori. In *L. digitata*, sori are identifiable as dark, slightly elevated areas on distal parts of the blade. Sori are composed of sterile paraphyses and sporangia, which produce and release haploid, flagellated meiospores (sporogenesis; Bold and Wynne, 1985; Bartsch et al., 2008, 2013). Despite their flagella and energy reserves for active swimming (Reed et al., 1999), meiospores are likely passively distributed and often settle on a suitable substrate close to their parental sporophyte (Schiel and Foster, 2006). Chemotaxis may facilitate the identification of substrate and spore settlement (Amsler and Neushul, 1989). Meiospores germinate into an initial gametophyte cell, and

grow into free-living, dioecious (independent male and female) gametophytes. These may persist vegetatively for months or even years if conditions do not allow fertility (e.g. inhibition by temperature or low irradiance; Lüning, 1980; tom Dieck, 1993; Edwards, 2000). Gametophytes produce gametes from haploid, somatic cells, whereby any gametophyte cell may develop into a gametangium. Male gametophytes produce spermatozoids in antheridia and female gametophytes produce eggs in oogonia (gametogenesis; Bold and Wynne, 1985; Martins et al., 2017). Egg release from the oogonium is induced by darkness (Lüning, 1981). Upon release, eggs stay loosely attached to the oogonium and produce the pheromone lamoxirene, which triggers sperm release from antheridia and attracts sperm to fertilize the eggs (Müller et al., 1985; Maier et al., 2001). Following fertilization, diploid zygotes develop into next-generation sporophytes.

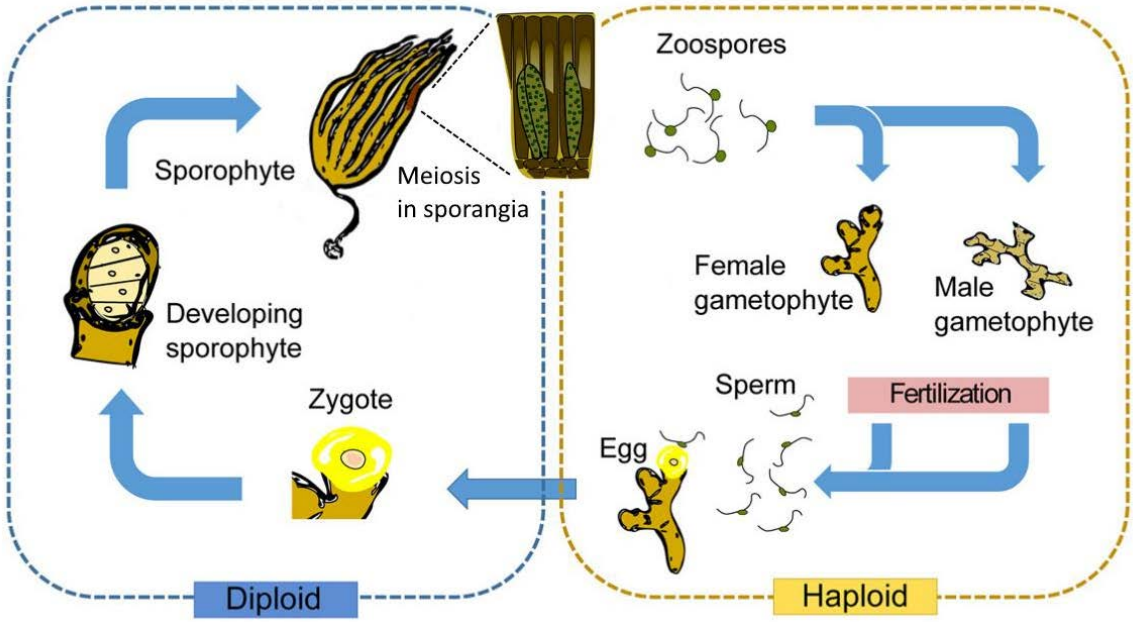

Figure 1.3 Schematic life cycle of *Laminaria digitata* modified from Lipinska et al. (2015); Visch et al. (2019). Source materials are published under license CC-BY 4.0.

In the biphasic kelp life cycle, the definition of a generation is not intuitive, especially when discussing generations in terms of parent and offspring. Often, the distinct gametophyte and sporophyte life cycle stages are described as "generations" (Coelho et al., 2007). However, these life cycle stages serve different functions in the reproductive cycle of kelps. Only the sporophyte produces meiospores and only the gametophyte produces gametes. The life cycle is completed once both stages have gone through their respective development. Therefore, in the context of this study investigating effects within and across gametophyte and sporophyte life cycle stages, I consider the gametophyte as an outsourced phase of the kelp germline. A germline is defined as the reproductive lineage of cells in an organism starting with the first cell which is committed to produce gametes

(Grossniklaus, 2011; Schmidt et al., 2015). In kelps, this definition encompasses all cells in the life cycle from the meiosporangium mother cell formed by the sporophyte in soral tissue to the oogonia and spermatozoids produced by female and male gametophytes, respectively. Apart from meiosis, the majority of the kelp germline is therefore contained in the autonomous gametophyte life cycle stage. According to this definition, gametophytes produce the gametes of a parental generation and the diploid zygote initiates the offspring generation.

1.3.3 Thermal characteristics of *Laminaria digitata*

Gametophytes and sporophytes of seaweeds often inhabit different thermal niches in terms of survival, growth and reproduction (Wiencke et al., 1994). In **Figure 1.4**, I integrated the available information on the thermal characteristics of *L. digitata*'s life cycle. To classify the available data on thermal responses of growth and reproductive traits (i.e. gametogenesis speed; recruitment; reproductive efficiency of sporogenesis *sensu* Bartsch et al., 2013), I arbitrarily defined temperatures eliciting 80–100% of maximum trait expression as the thermal optimum, 50–80% as sub- and supraoptimum, and < 50% as the tolerance range. *L. digitata* gametophytes survive at temperatures from -1.5 to 23°C for two weeks (Bolton and Lüning, 1982; tom Dieck, 1993; Wiencke et al., 1994). The upper survival temperature of laboratory-cultivated *L. digitata* sporophytes was determined at 21°C over two weeks, 2°C lower than that of gametophytes (Bolton and Lüning, 1982; tom Dieck, 1992). The cold survival temperature of sporophytes has not been determined experimentally to my knowledge, but their occurrence in the Arctic suggests a cold thermal tolerance limit < 0°C (van den Hoek, 1982b). Primary gametophyte cells and multicellular gametophytes grow optimally at 10–18°C over one week (Lüning, 1980; Martins et al., 2017). Vegetative gametophyte growth is retained at 20°C but strongly inhibited at 22°C (pers.

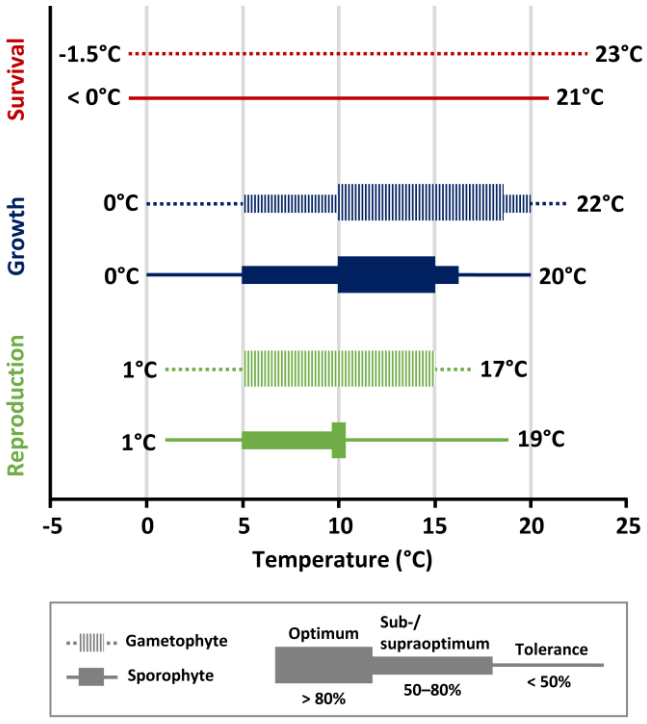

Figure 1.4 Thermal characteristics of survival (red), growth (blue) and reproduction (green) of *Laminaria digitata* gametophytes (dotted lines) and sporophytes (solid lines). Limits describe minimum and maximum temperatures at which *L. digitata* survives, grows and reproduces. Classification with respect to maximum trait response of growth and reproduction: > 80%, optimum; 50–80%, sub-/supraoptimum; < 50% tolerance range. For details and references, see main text.

obs.; see also Martins et al., 2020). Sporophytes achieve optimum growth at 10–15°C and grow suboptimally at temperatures ≥ 5°C over at least two weeks, whereas 0 and 20°C delimit the thermal growth range (Perez, 1971; Bolton and Lüning, 1982; tom Dieck, 1992; Wilson et al., 2015). Optimum temperatures for gametophyte reproduction range between 5 and 15°C, depending on the observed trait (Lüning, 1980; Martins et al., 2017). Gametogenesis is faster at 10–15°C than at 5°C (Martins et al., 2017), but the number of recruited juvenile sporophytes was twice as high at 5°C compared to 15°C under laboratory conditions (Martins et al., 2017). Prolonged vegetative growth at 5°C may produce more female gametophyte cells (pers. obs.) which all have potential to develop into oogonia. Alternatively, the slower development at 5°C presumably allows for several events of gamete release which may optimize fertilization success (Martins et al., 2017). Therefore, reduced gametogenesis speed may be interpreted as an adaptive response increasing recruitment at temperatures which are suboptimal for the process of gametogenesis (Bolton and Levitt, 1985). Gametogenesis is strongly inhibited at 0°C (Sjøtun and Schoschina, 2002) and 18°C (Martins et al., 2017). Reproductive efficiency of *L. digitata* sporophytes is optimal at 10°C, as was shown by Bartsch et al. (2013), who integrated parameters of sorus occurrence, sorus area and reaction time of sporogenesis. Sporogenesis is delayed but not inhibited at 1 and 5°C, whereas at 15–19°C occurrence and size of sori progressively decrease. Therefore, sporogenesis is more efficient at 1°C than at ≥ 16°C (Bartsch et al., 2013). Like gametogenesis, sporogenesis occurs at up to 17°C, but is severely inhibited at temperatures ≥ 18°C (tom Dieck, 1992; Bartsch et al., 2013; Martins et al., 2017).

In the wild, phenology of *L. digitata* is controlled by several abiotic and biotic factors in addition to temperature. First, the pattern of high growth from February to July and low growth from August to January (Perez, 1971; Kain, 1979) is in part controlled by an endogenous rhythm, which is synchronized by seasonal day length cues (Schaffelke and Lüning, 1994; Gomez and Lüning, 2001). *L. digitata* sporophytes can potentially carry sori year-round (Cosson, 1976; Chapman, 1984), but the main fruiting period occurs during summer to late autumn when growth is reduced (Bartsch et al., 2008). The meristem is hypothesized to export a substance inhibiting sorus formation during phases of active growth (Lüning et al., 2000). A candidate substance is the auxin hormone indole-acetic acid which was shown to inhibit sorus formation in *Saccharina japonica* (Kai et al., 2006). Therefore, the onset of fertility of *L. digitata* sporophytes is probably controlled by the seasonal growth rhythm, which itself is controlled by the photoperiod (Bartsch et al., 2013). Temperature, among other factors (e.g. nutrients; Nimura et al., 2002), may then modulate the speed and efficiency of sporogenesis. Following settlement and germination of the released meiospores, the majority of gametophytes is believed to reproduce in late autumn when temperatures decrease and before low irradiance becomes limiting (Lüning, 1980; Sjøtun and Schoschina, 2002; Martins et al., 2017). Kelp gametogenesis is modulated by photoperiod, intensity of irradiance, the irradiance spectrum, nutrient availability and temperature. For instance, gametogenesis is inhibited under red light (Lüning and

Dring, 1975) and lack of bioavailable iron (Iwai et al., 2015). For *L. digitata*, gametogenesis was maximal under low irradiance of white light in a long photoperiod at high nutrient concentrations (Martins et al., 2017). Due to their potential for perennial vegetative growth (tom Dieck, 1993; Edwards, 2000), gametophytes may recruit juvenile sporophytes year-round when conditions become favourable.

L. digitata occurs between the 0°C winter and 18–19°C summer mean sea-surface isotherms, beyond which it is most likely limited by insufficient growth or reproduction (van den Hoek, 1982a, 1982b; Müller et al., 2009; Oppliger et al., 2014). The distribution of *L. digitata* in the Northeast Atlantic is predicted to shift northward during ocean warming (Raybaud et al., 2013; Assis et al., 2018). This implies an extinction of the southernmost populations from Brittany, France, to the North Sea, and an expansion along Arctic coasts. Such predictions are often based on niche models which assume that all individuals within a species perform uniformly (Müller et al., 2009; Reed et al., 2011; King et al., 2018). However, intraspecific trait variability is an important aspect of a species' environmental niche and can, if implemented, improve and change predictions (Cacciapaglia and van Woesik, 2018; Bennett et al., 2019; Chardon et al., 2020). Especially local adaptation and phenotypic plasticity have been shown to affect species' responses to climate change (Atkins and Travis, 2010; Valladares et al., 2014; Bennett et al., 2019), and are part of a framework of trait variability which potentially affects responses of species from levels of populations to individuals.

1.4 Thermal trait variability within a species

Within the thermal niche of a species, different levels of intraspecific trait variability can be classified along scales of space and time. Here, I introduce relevant concepts which have been described to modulate trait expression within species, in part including kelp. I illustrate theoretical concepts of thermal trait variability in schematic diagrams of thermal performance (**Figure 1.5–Figure 1.8**) which are loosely based on thermal growth curves of *L. digitata* sporophytes (Bolton and Lüning, 1982; tom Dieck, 1992). On these concepts, I based my research questions and hypotheses (**Chapter 1.5**) for the comprehensive investigation of central response mechanisms of kelp using the example of *L. digitata*. All relevant definitions are compiled in the **Glossary** (core concepts are marked bold).

Along environmental gradients on a large spatial scale, populations of the same species may experience local **adaptation** to their prevailing environment due to natural selection (**Figure 1.5**; Kawecki and Ebert, 2004; Sanford and Kelly, 2011). Changes in allele frequencies alter a population's ecological niche towards a local optimum, which optimizes its physiology in the local habitat only (Kawecki and Ebert, 2004). Locally adapted varieties of a species are called ecotypes (Gregor, 1944; King et al., 2018). In theory, local adaptation is favoured in populations with high genetic diversity for natural selection to target, and low gene flow which does not counteract selective

forces (Antonovics, 1976; Kirkpatrick and Barton, 1997). Populations at a species' ecological (and often geographical) range edges are of special interest, because they experience the most extreme conditions tolerable by the species (marginal populations *sensu* Soulé, 1973). If these unfavourable environments reduce population size and genetic diversity (Kawecki, 2000; Eckert et al., 2008), genetic drift may lead to **maladaptation** (Crespi, 2000; Eckert et al., 2008; Pearson et al., 2009). In contrast, extant *L. digitata* populations close to their southern distribution limit in Brittany, France, harbour high and unique genetic diversity (Oppliger et al., 2014; Robuchon et al., 2014b; Neiva et al., 2020), likely due to the persistence of refugial populations in the Armorican/Celtic Sea during the Last Glacial Maximum (Assis et al., 2018; Neiva et al., 2020). Strong selective forces acting on these unique populations might, in contrast, have facilitated local adaptation (Hardie and Hutchings, 2010). A recent meta-analysis found evidence for intraspecific local thermal adaptation in 90% of the investigated studies on macroalgae and seagrass (King et al., 2018). Local adaptation might be favoured in these macrophytes due to their low dispersal capacity and low gene flow among populations (Breeman and Pakker, 1994; King et al., 2018; Miller et al., 2019). Recent evidence for local adaptation among *L. digitata* populations shows maximum production of heat shock proteins at higher temperature in Southern English populations at the distributional trailing edge, compared to Scottish populations in the species' range centre (King et al., 2019). Additionally, *L. digitata* gametophytes from the Arctic and the North Sea showed slight thermal differentiation in terms of growth and fertility in accordance with their local thermal regimes (Martins et al., 2020). In the sugar kelp *Saccharina latissima*, ecotypic differentiation of thermal tolerance has been described among Northwest Atlantic populations decades ago (Gerard and Du Bois, 1988), and current transcriptomic analysis of responses to heat and hyposalinity corroborated differentiation among Brittany and Arctic populations (Monteiro et al., 2019b). However, studies on thermal traits within and among kelp populations on broad geographical scales are not yet sufficient to assess the role of local adaptation in shaping species' responses to ocean warming (Nepper-Davidsen et al., 2019).

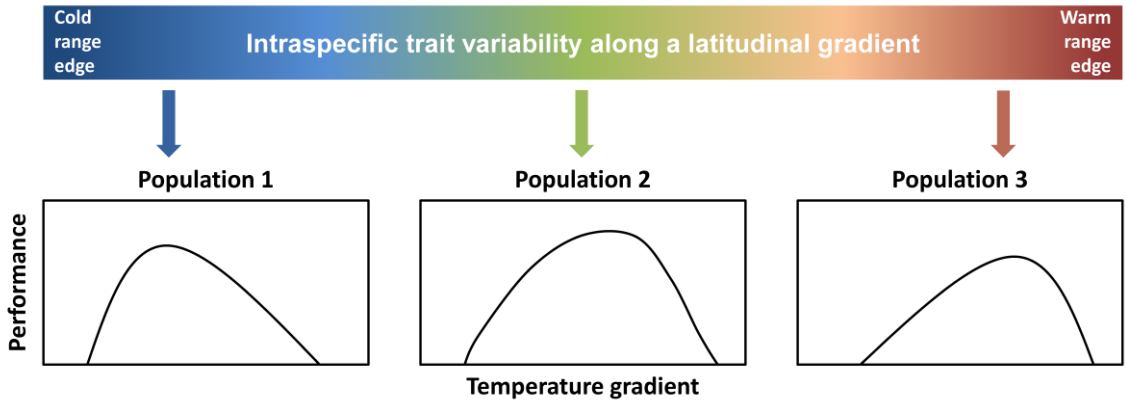

Figure 1.5 Concept of hypothetical intraspecific trait variability of a kelp species over a temperature gradient. **I.** Along a latitudinal gradient, populations may differ in their average thermal performance (i.e. reaction norms differing in shape and/or thermal limits, e.g. of growth) due to **local adaptation** to the prevailing conditions.

On a smaller spatial scale, individual trait variability plays an important role in shaping the average response of populations in general (**Figure 1.6**; Sultan, 1995; Reusch, 2014). **Phenotypic plasticity** describes the ability of one genotype (i.e. the entirety of genes within an individual) to produce several phenotypes in response to its environment (Nicotra et al., 2010), which can be displayed as a reaction norm of a trait over an environmental gradient. This is the basis of acclimation, by which organisms can quickly adjust their metabolism to enhance or maintain performance in response to an environmental change (Reusch, 2014). *L. digitata* displays a wide thermal performance range of more than 15°C, in which plastic responses are capable of maintaining all essential life cycle functions (see **Chapter 1.3.3** and **Figure 1.4**). The shape of reaction norms may also differ among genotypes, in that they differ in their capacity to express plastic traits. This concept is termed **genetic variation for plasticity** (Newman, 1994) and can be quantified by assessing significant differences among genotypes in their phenotypic response to an environmental gradient (genotype x environment interaction; Saltz et al., 2018). Genetic variation for plasticity implies that plasticity of fitness-related traits itself can be a trait that natural selection targets, thereby allowing reaction norms to evolve (Newman, 1994). For instance, a variable environment may select for high trait plasticity if some resulting phenotypes are of higher fitness than the consistent phenotype produced by a non-plastic genotype (Newman, 1994). Gametophytes of the giant kelp *Macrocystis pyrifera* responded differently among lineages to irradiance and temperature in growth and fertility characteristics (Mabin et al., 2019), which is a first indication of genetic variation for plasticity in a kelp. However, the prevalence and magnitude of genetic variation for plasticity in kelps is yet unclear.

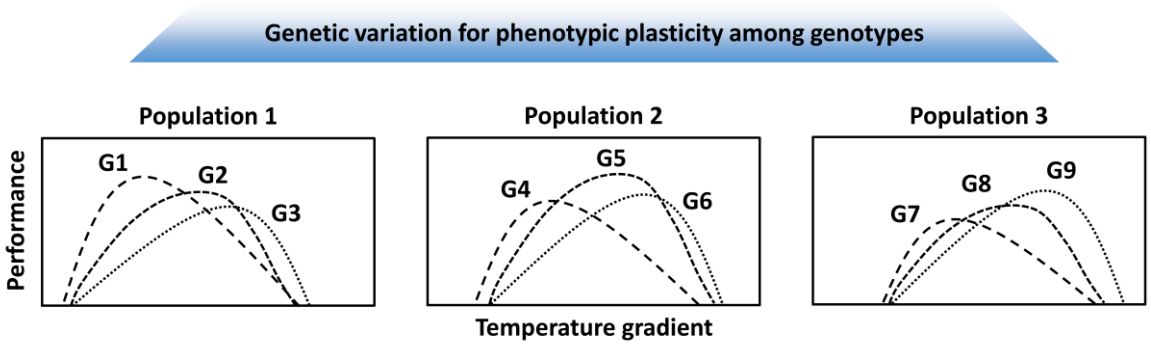

Figure 1.6 Concept of hypothetical intraspecific trait variability of a kelp species over a temperature gradient. **II.** Within a population, genotypes (G1–G9) express **phenotypic plasticity** as the capacity to produce a range of different phenotypes over the temperature gradient. **Genetic variation for plasticity** describes differential phenotypic plasticity among genotypes and is visualized as different reaction norm shapes and thermal ranges. Together, these reaction norms form the average population response (**Figure 1.5**).

Even within a genotype, reaction norms may not be static. The expression of different phenotypes by a genotype depending on the environment may affect its traits at a later time in **carry-over effects** (COE, also developmental plasticity; **Figure 1.7**; Palmer et al., 2012; Byrne et al., 2020). For instance, blade length of *Ecklonia radiata* sporophytes was correlated to the amount of reproductive tissue produced (Mabin et al., 2013). This indicates that the environment shaping sporophyte growth also affected their reproductive capacity at a later time. Additionally, trait expression may be shaped across life cycle stages and/or generations. **Parental effects** describe an influence of the parental environment on offspring traits regardless of the offspring environment (*sensu* Salinas et al., 2013; **Figure 1.7**). Parental effects produce parallel reaction norms among offspring of parents from different environments, which only differ in their magnitude of trait expression. In **cross-generational plasticity** (CGP; **Figure 1.7**; Byrne et al., 2020), an interaction of parent and offspring environments changes the shape of the offspring reaction norm (i.e. its plasticity). Evidence for an effect of the parental environment on offspring traits exists for the fucoid seaweed *Fucus vesiculosus*, in which cultivation of receptacles during gametogenesis at 14°C compared to 4°C increased survival of subsequently recruited embryos at 33°C for 3 h from 36% to 64% (Li and Brawley, 2004). This indicates that ontogenetic temperature history may potentially have important effects on thermal plasticity and resilience to warming also in kelp forest key species. Suspected drivers of these effects are non-genetic mechanisms such as gene methylation, chromatin modification, energy transfer or hormone signalling (e.g. reviews by Jablonka and Raz, 2009; Ho and Burggren, 2010). Experiments addressing effects across generations are often designed as full-factorial approaches, in which offspring from two parental treatments are tested in contrasting environments either matching or mismatching their parental environment (Engqvist and Reinhold, 2016; Donelson et al., 2018).

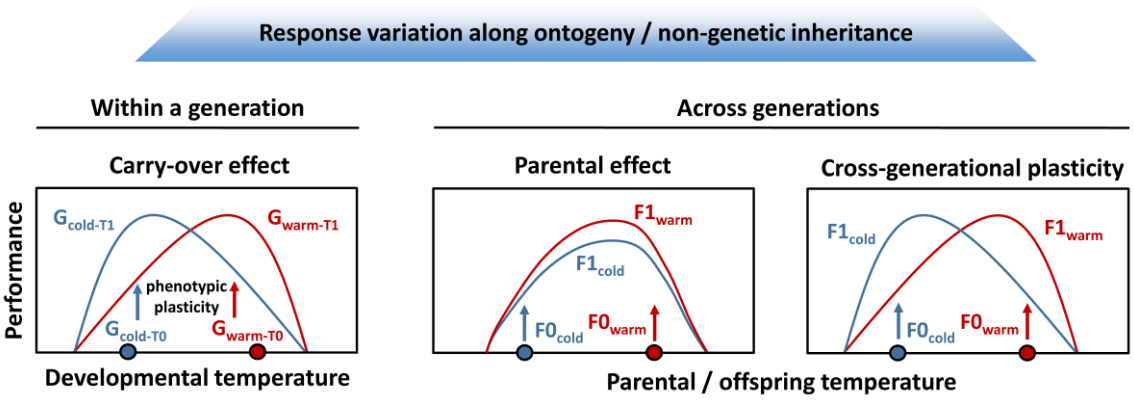

Figure 1.7 Concept of hypothetical intraspecific trait variability of a kelp species over a temperature gradient. **III. Carry-over effects** occur **within a generation**. Early development of a genotype (G) in a specific environment (blue and red dot; T0) affects trait expression (i.e. shape and/or elevation of the reaction norm) of the same genotype later in time (T1) due to development of different phenotypes. **Across generations**, **parental effects** of a temperature experienced by the parent (F0; blue and red dot) evoke a general benefit or disadvantage in the offspring (F1) regardless of offspring environment (i.e. parallel reaction norms with similar shape). **Cross-generational plasticity** alters offspring plasticity (i.e. shape of the reaction norm) across generations in an interaction of environments experienced by parents (F0; blue and red dot) and offspring (F1).

Lastly, **outbreeding** among differentiated genotypes may produce new phenotypes (**Figure 1.8**; Birchler et al., 2010; Westermeier et al., 2010). Results from a heat experiment on interspecific hybrids among *L. digitata* and *L. pallida* (Martins et al., 2019) allow the assumption that thermal tolerance limits may be inherited from the female parent, but this is not a consistent pattern in interspecific kelp crosses (tom Dieck and de Oliveira, 1993). Additionally, hybrid offspring sporophytes of *L. digitata* and *L. pallida* had a higher thermal tolerance compared to either of their single-species parental lineages (Martins et al., 2019). This effect of increased performance of hybrid offspring is known as **heterosis** or hybrid vigour (Birchler et al., 2010). In mid-parent heterosis, the hybrid performs intermediate to its parental lineages, but significantly better than the average performance over both parental lineages (Hochholdinger and Hoecker, 2007). In best-parent heterosis, hybrid performance surpasses the better of the two parents (Hochholdinger and Hoecker, 2007). Within a species, genetic and phenotypic variation among populations may produce new phenotypes if ecotypes of the same species are outbred. Generally, outbreeding among populations may alleviate negative effects of genetic drift and inbreeding depression by introducing new alleles to a genetically impoverished population's gene pool (Charlesworth and Charlesworth, 1987). In contrast, outbreeding depression may occur as a disruption of local adaptation among crosses of differentiated populations which may reduce their performance and fitness (Waser and Price, 1989; McKay et al., 2005; Aitken and Whitlock, 2013). Heterosis has been shown in improved growth and fertility of outbred compared to inbred *Macrocystis pyrifera* sporophytes (Raimondi et al., 2004; Westermeier et al., 2010) and in seaweed cultivars in mariculture (Li et al., 2007, 2008). However, the mechanisms of thermal inheritance and heterosis are still poorly understood for kelps (Westermeier et al., 2010; Martins et al., 2019).

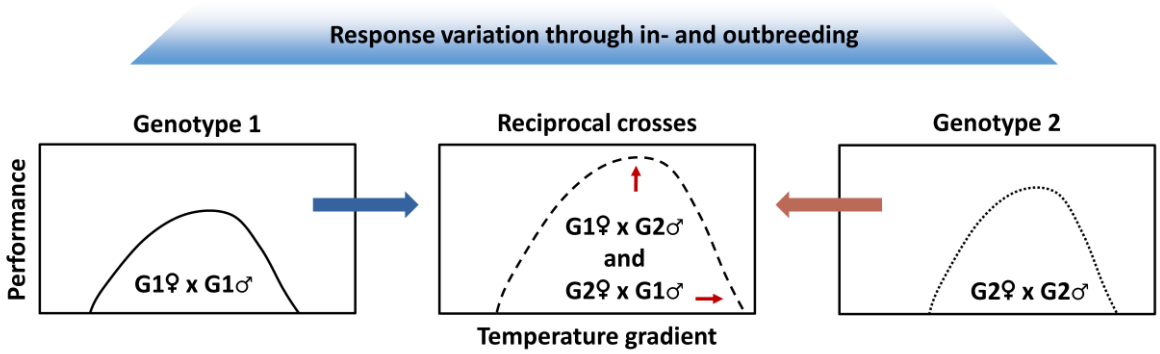

Figure 1.8 Concept of hypothetical intraspecific trait variability of a kelp species over a temperature gradient. **IV.** New phenotypes may be produced by **outbreeding** among genotypes with differentiated traits (G1, G2). The exemplary reaction norm for the reciprocal crosses indicates **best-parent heterosis** for maximum trait performance and heat tolerance (indicated by red arrows).

1.5 Objectives and research questions

Trait variability via phenotypic plasticity and genetic variation is an important means of reacting to environmental change, the principles of which have been researched for decades (Bradshaw, 1965; Via and Lande, 1985). General research on phenotypic plasticity is still of utmost actuality today (Nicotra et al., 2010; Saltz et al., 2018; Fox et al., 2019). Trait variability is especially important for sessile species to acclimate and/or adapt to a changing environment and to avoid range shifts. Distribution ranges of marine forests are predicted to shift poleward along with rising ocean temperatures (Raybaud et al., 2013; Assis et al., 2018), and first losses of trailing-edge seaweed and kelp populations are already documented worldwide (e.g. Nicastro et al., 2013; Wernberg et al., 2016; Assis et al., 2017). However, this recent focus of ecological kelp research on trailing-edge populations contrasts the lack of knowledge on species responses to ocean warming on a broad scale along environmental gradients (Nepper-Davidsen et al., 2019; King et al., 2020b). Understanding the processes driving differential responses of kelps to ocean warming will allow us to estimate the resilience, flexibility and adaptability of marine forest key species, and to improve predictions and mitigation guidelines. Further, identification of mechanisms mitigating ocean warming through either natural or artificial measures will provide a strong tool for bioconservation (Filbee-Dexter and Smajdor, 2019; Coleman et al., 2020b). It is still mostly unknown if and to what extent thermal performance and tolerance differ among populations of kelp species (but see e.g. Martinez, 1999; King et al., 2019), or if thermal ecotypes are capable of buffering predicted losses at the warm range edge due to warming. Further, it is unknown if temperature experienced during ontogeny may induce acclimative responses later in the life cycle (as in *F. vesiculosus*; Li and Brawley, 2004), providing a potential non-genetic mechanism to quickly respond to environmental change. Lastly, potential differentiation among kelp populations may provide a useful model to investigate thermal inheritance and effects of outbreeding among differentiated lineages on the thermal resilience within a kelp species (as in *M. pyrifera*; Westermeier et al., 2010). If these concepts are integrated into a theoretical framework, this will produce a comprehensive overview of the thermal responses and trait variability within one species which complements fundamental thermal optimum curves (e.g. Bolton and Lüning, 1982).

The overarching objective of this dissertation was thus to produce an assessment of thermal trait variability of a marine forest key species across populations and life cycle stages for the first time in such detail. I investigated thermal characteristics of the kelp *Laminaria digitata* among populations covering the entire Northeast Atlantic distribution range (**Publication I**), along ontogeny over life cycle stages of gametophyte and sporophyte (**Publication II**), and among inbred and outbred sporophytes from contrasting temperature environments (**Publication III**). Specifically, I designed three studies to answer the following major research questions:

Research question of Publication I

Have populations of *Laminaria digitata* differentiated in their heat resilience along the species' entire Northeast Atlantic latitudinal distribution?

Meristematic samples of *L. digitata* sporophytes were collected in Spitsbergen, Norway (79°N); Tromsø, Norway (70°N); Bodø, Norway (67°N); Helgoland, Germany (54°N); Roscoff, France (49°N); and Quiberon, France (47°N). Samples were subjected to a mechanistic common garden experiment applying 15–23°C over eight days to identify differences in response to sublethal heat in growth, chlorophyll fluorescence, carbon and nitrogen storage, and pigment contents. To investigate mechanisms facilitating potential ecotypic differentiation, neutral microsatellite markers were used for population genetic analyses of connectivity and genetic diversity.

I hypothesized that *L. digitata* populations at the northern distribution limit are less heat-tolerant than populations at the southern distribution limit (i.e. thermal ecotypes have differentiated).

I hypothesized that differentiation occurred in populations experiencing low amounts of gene flow, and that low genetic diversity was associated with reduced heat resilience due to genetic drift and possible maladaptation.

Research question of Publication II

Does ontogenetic temperature history across life cycle stages alter thermal plasticity of juvenile *Laminaria digitata* sporophytes?

Five distinct genetic lines were initiated by separately releasing meiospores from five wild *L. digitata* sporophytes from Helgoland. Genetic lines were cultivated at the contrasting temperatures of 5 and 15°C in a full-factorial approach across three steps (1) from meiospore germination over gametogenesis to recruitment of offspring sporophytes, (2) rearing of juvenile sporophytes and (3) a 12-day experiment assessing thermal plasticity of 3–4 month-old offspring sporophytes. By splitting each genetic line into treatments of 5 and 15°C for each step, this created a total of eight temperature history treatments, in which I assessed the response parameters growth, chlorophyll fluorescence, and carbon and nitrogen storage. To relate results of thermal plasticity to seasonality in the wild, I conducted a separate growth experiment at 5 and 15°C with meristematic material from wild sporophytes across seasons.

I hypothesized that the temperature experienced during gametogenesis and recruitment increases performance of juvenile sporophytes at matching temperatures (i.e. carry-over or cross-generational effects).

I hypothesized that thermal plasticity is not uniform, but differs among genetic lines (i.e. genetic variation for plasticity).

Research question of Publication III

How are thermal traits inherited among cold-temperate and Arctic *Laminaria digitata*?

Unialgal female and male gametophyte isolates from Helgoland, North Sea, and Spitsbergen, Norway were used to rear lineages of inbred, within-population selfings and outbred reciprocal crosses of *L. digitata* sporophytes. In a first experiment, I assessed the upper temperature tolerance of inbred and outbred microscopic sporophytes over two weeks. In a second experiment, I subjected macroscopic sporophytes to 10°C (control) 19°C (moderate heat) and 20.5°C (sublethal heat) for 18 days, during which I measured growth and chlorophyll fluorescence. At the end of the experiment, transcriptomic samples were analysed for differential gene expression among lineages to identify metabolic pathways involved in heat responses, and to estimate cellular stress responses expressed to maintain physiological performance.

I hypothesized that sporophytes produced by inbreeding (selfing) Helgoland *L. digitata* are more tolerant to sublethal high temperature than inbred Spitsbergen sporophytes due to ecotypic differentiation of the respective populations.

I hypothesized that outbred hybrids (crosses) among populations perform intermediate to or better than the selfings (i.e. mid-parent or best-parent heterosis).

1.6 List of publications and declaration of own contribution

Publication I

Daniel Liesner, Louise Fouqueau, Myriam Valero, Michael Y. Roleda, Gareth A. Pearson, Kai Bischof, Klaus Valentin, Inka Bartsch (2020). **Heat stress responses and population genetics of the kelp *Laminaria digitata* (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations.** *Ecology and Evolution*, 10: 9144–9177. doi:10.1002/ece3.6569.

The experimental design of this study was developed as a common protocol in the EU-BiodivERsA project MARFOR under the lead of I. Bartsch, supported by M. Valero, G. A. Pearson, M. Y. Roleda and D. Liesner. Sampling of field material was conducted by D. Liesner, L. Fouqueau, M. Y. Roleda and I. Bartsch. Experiments and data acquisition were conducted by D. Liesner with support from I. Bartsch (experiment with Spitsbergen material) and K. Bischof (pigment analysis). Analysis and presentation of physiological data was conducted by D. Liesner; analysis and presentation of population genetic data was conducted by L. Fouqueau and M. Valero. D. Liesner wrote the manuscript draft with support from L. Fouqueau and M. Valero, who provided drafts for the material and methods and results chapters for population genetic analyses. All co-authors discussed and revised the manuscript under the lead of Daniel Liesner.

Contribution of the candidate in % of the total workload (up to 100% for each category):

Experimental concept and design:	ca. 50%
Experimental work / data acquisition:	ca. 80%
Data analysis and interpretation:	ca. 80%
Preparation of Figures and Tables:	ca. 80%
Drafting of the manuscript:	ca. 85%

Publication II

Daniel Liesner, Lisa N. S. Shama, Nora Diehl, Klaus Valentin, Inka Bartsch (2020). **Thermal plasticity of the kelp *Laminaria digitata* (Phaeophyceae) across life cycle stages reveals the importance of cold seasons for marine forests.** *Frontiers in Marine Science*, 7: 456. doi:10.3389/fmars.2020.00456.

D. Liesner developed the concept of this manuscript with L. N. S. Shama and I. Bartsch. Sampling was conducted by D. Liesner and I. Bartsch. Experiments and data acquisition were conducted by D. Liesner with support in chlorophyll fluorescence analysis by N. Diehl. Analysis and presentation of physiological data was conducted by D. Liesner with support from L. N. S. Shama and N. Diehl. D. Liesner wrote the manuscript draft. All co-authors discussed and revised the manuscript under the lead of D. Liesner.

Contribution of the candidate in % of the total workload (up to 100% for each category):

Experimental concept and design:	ca. 80%
Experimental work / data acquisition:	ca. 80%
Data analysis and interpretation:	ca. 90%
Preparation of Figures and Tables:	100%
Drafting of the manuscript:	ca. 99%

Publication III

Daniel Liesner, Shivani Rana, Lars Harms, Inka Bartsch, Gernot Glöckner, Klaus Valentin (in preparation). **Evidence for increased heat resilience of intraspecific hybrids compared to inbred lineages of the kelp *Laminaria digitata* (Phaeophyceae) in physiology and transcriptomics.**

Authors to be included before manuscript submission: Gareth Pearson, Sandra Heinrich, Kai Bischof. Journal to be decided.

D. Liesner developed the concept of this manuscript with I. Bartsch, K. Valentin and G. Glöckner. Experiments and data acquisition were conducted by D. Liesner. Physiological data analysis and presentation was conducted by D. Liesner. G. Glöckner provided the reference transcriptome and D. Liesner performed analyses of differential gene expression with support from L. Harms. D. Liesner wrote the manuscript draft with support from G. Glöckner and S. Rana, who provided information for the bioinformatic materials and methods chapter. The manuscript was discussed and revised with G. Glöckner, K. Valentin and I. Bartsch, and will be finalized with all co-authors before submission.

Contribution of the candidate in % of the total workload (up to 100% for each category):

Experimental concept and design:	ca. 60%
Experimental work / data acquisition:	ca. 80%
Data analysis and interpretation:	ca. 80%
Preparation of Figures and Tables:	100%
Drafting of the manuscript:	ca. 95%

2 Synopsis of methodology

2.1 The kelp experimental system – benefits and disadvantages

To investigate intraspecific trait variability, kelps provide an intriguing experimental system. The structuring of sporophytes into a complex thallus allows to, within limits, infer whole-organisms responses from experiments on isolated tissue. For instance, samples of kelp and fucoid tissue can be used to examine growth responses of meristematic material (Graiff et al., 2015), chlorophyll fluorescence (Hargrave et al., 2017), fertility (Bartsch et al., 2013), transcriptomic stress responses (King et al., 2019), and even allow investigation of traits along the basal-distal gradient of kelp blades (Buchholz and Lüning, 1999; Scheschonk et al., 2019). However, results have to be interpreted with caution, as the isolation of tissue samples intercepts any basal-distal relationships in the thallus. Examples for such relationships are the hypothesized control of sporogenesis in the distal blade via auxin hormones produced by the meristem (Buchholz and Lüning, 1999; Lüning et al., 2000; Kai et al., 2006), or the reversible transformation and translocation of the storage carbohydrates mannitol and laminarin (Yamaguchi et al., 1966; Gómez and Huovinen, 2012; Scheschonk et al., 2019). In the context of this study, the experimental use of meristematic tissue samples allowed to compare responses of wild sporophytes along the latitudinal distribution of *L. digitata* in replicated, common garden experiments (**Publication I**). Tissue discs were cut from the sporophyte meristem and stored within wet paper towels in ziplock bags. At cool temperature (< 15°C), these samples can be stored and transported within 30 hours without evident damage, to be used in a laboratory experiment.

Gametophytes, on the other hand, provide benefits because of their small size and separate sexes. Unialgal clonal gametophyte cultures can be prepared and maintained with relatively low effort and can be fertilized at will to produce sporophytes (Bartsch, 2018). This quality may, for instance, be applied in producing gametophytes as seeding material for restoration efforts (Fredriksen et al., 2020; Vanderklift et al., 2020). As discussed above, gametophytes may be viewed as constituting the majority of the kelp germline as they produce gametes, and are therefore part of the parental generation producing the next generation of offspring sporophytes (see **Chapter 1.3.2**). This allows to experimentally alter the environment during gametogenesis uncoupled from the parental somatic cells, of which I made use in investigating the effects of temperature across kelp generations and ontogeny (**Publication II**). However, when investigating cross-generational effects, it is important to separate experimental treatments between both generations to clearly attribute effects to the treatment of one generation (Donelson et al., 2018; Byrne et al., 2020). In laminarian kelps, a separation of treatments during gametogenesis, but excluding next-generation sporophytes is difficult. The timing of female and male gamete release is co-dependent (Maier et al., 2001) and occurs over several days (Lüning, 1981) and as yet there are no feasible methods to fully inhibit and induce gamete release, or to isolate gametes for controlled fertilization. A revised method to circumvent an overlap of generational treatments is to treat only vegetative gametophytes experimentally and induce gametogenesis and fertilization in a common environment (**Chapter**

6.1.3; Gauci, 2020). I conceptualized this experimental design as part of a Master's thesis which I co-supervised (Gauci, 2020) following **Publication II** (see **Chapter 6.1.3**). In this approach, only primordial germ cells, but not gametes and zygotes are exposed to the experimental treatment, and any treatment effects can be attributed solely to the parental gametophyte environment. If taken one step further, the kelp experimental system also allows to treat only parental diploid sporophyte material (e.g. distal sporophyte discs; Bartsch et al., 2013), and investigate cross-generational effects on next-generation sporophytes across meiosis and gametogenesis. This approach circumvents an issue common in animal models, that germ cells and embryos cannot easily be separated from their parents (Torda et al., 2017; Donelson et al., 2018; Byrne et al., 2020).

Gametophytes originating from meiospores of one sporophyte can further be used to produce selfings as biological replicates (**Publication II**). Clonal gametophyte cultures can even be used to produce multiple genetically identical offspring sporophytes (**Publication III**; Westermeier et al., 2010), which allows investigations of phenotypic plasticity by differentially treating multiple individuals of one genotype. However, true homozygous kelp lines are difficult to produce as they have to be repeatedly inbred over multiple generations. This is a standard procedure to obtain stable cultivars in kelp mariculture (e.g. Li et al., 2008), but poses an issue in controlled laboratory settings. Between fertilizing gametophyte material and having reared macroscopic sporophytes suited for experiments, at least three months of cultivation are necessary (Bartsch, 2018), while the natural life cycle is completed within ~1 year. For long-term cultivation or to complete the kelp life cycle under laboratory conditions, either seawater tank systems are necessary (e.g. Schaffelke and Lüning, 1994) or sporophytes may be kept in large beakers with frequent seawater exchanges for several months (Bartsch et al., 2013), which poses an immense logistic effort. In comparison, the small, filamentous brown alga *Ectocarpus siliculosus* has meanwhile become a model organism to study evolution and developmental processes at the molecular level (Coelho et al., 2007; Cock et al., 2010, 2014), partly because of its small size and quick generational turnover (Peters et al., 2004). Still, kelps offer a valuable experimental system with unique characteristics and potential for fundamental research, ecosystem conservation and applied mariculture.

2.2 Experimental designs

In **Publication I**, I conducted a common garden experiment designed around the upper thermal tolerance limit of *L. digitata* sporophytes to test for differentiation in heat tolerance among populations. For this, meristematic samples from five locations were tested in a laboratory experiment under identical conditions. To identify true local adaptation to the entirety of a local environment, individuals from different locations should be transplanted reciprocally to compare fitness parameters at each location (Kawecki and Ebert, 2004). Ecotypes will perform comparatively

better in their respective environment, but worse in others. However, to investigate phenotypic differentiation in response to exclusive parameters (and not the integrative local environment), common garden experiments provide an ideal experimental design, while preventing any ethical conflicts due to genetic contamination among locations (King et al., 2018). Results obtained from wild material have to be interpreted with caution due to potential interaction of genetic effects (e.g. adaptation) with within- and cross-generational plasticity due to the samples' environmental history (King et al., 2018). These effects can only be ruled out by investigating laboratory-reared individuals over multiple generations, which was not feasible in the scope of this dissertation.

In **Publication II**, I investigated temperature effects across life cycle stages and ontogeny on thermal plasticity of juvenile sporophytes. I applied a full-factorial design tracking ontogeny of *L. digitata* at 5 and 15°C in three steps from meiospores to sporophytes. In the first step, I applied 5 and 15°C during meiospore germination, gametogenesis and sporophyte recruitment. Each treatment was then split into treatments of 5 and 15°C to be reared during early sporophyte growth. In a final step, treatments were again split between 5 and 15°C for an experiment assessing thermal plasticity of juvenile sporophytes among the, in total, eight temperature history treatments. Therefore, the experiment lacked a common control treatment, but comparisons among treatments allowed to assess the effect of matching or mismatching environments across ontogeny and development (match-mismatch approach; Engqvist and Reinhold, 2016). Further, the experiment was replicated in five distinct genetic lines each initiated from meiospores of one wild sporophyte individual. This allowed to disentangle genetic effects from environmental treatment effects, and made assessments of genetic variation for plasticity possible (Herman and Sultan, 2016; Donelson et al., 2018).

In **Publication III**, I used female and male gametophyte isolates, each obtained from one sporophyte individual from the Arctic archipelago of Spitsbergen and one from the North Sea island of Helgoland, to produce sporophytes of inbred selfings and outbred reciprocal crosses among the two populations. These sporophytes were then assessed in terms of their thermal resilience and transcriptomic profiles to identify effects of inbreeding and outbreeding, and to assess patterns of thermal inheritance among inbred and outbred lineages.

2.3 Physiological measurements

In all three publications, I investigated physiological characteristics of *L. digitata* sporophytes in thermal experiments based on established methods. This provided an integrative overview of key traits in kelp ecophysiology.

Growth is an integrative parameter over all metabolic processes and can therefore be interpreted as a proxy for organismal stress. In algae, growth is often described based on one of three characteristics: development of thallus length, area or mass over time (e.g. Lüning, 1990; Graiff et

al., 2015; Martins et al., 2019). Measurements are simple in that samples are removed from experimental beakers, photographed with a defined reference area, and patted dry and weighed on a laboratory scale. Images can then be analysed for thallus length and area with image analysis software. Length, as a one-dimensional characteristic, mostly increases linearly over time and can therefore often be described with absolute growth rates (e.g. as an increase in cm d^{-1}). In contrast, area and mass of sporophytes mostly increase exponentially over time due to their expansion in multiple dimensions. Therefore, area and mass growth over time is better expressed as relative growth rates (e.g. as an increase in g g^{-1} d^{-1} or % d^{-1}; Lüning, 1990). In **Publication II**, I compared temperature effects on length growth of sporophytes with differing initial sizes, which did have an effect on growth rates. To visualize solely the effect of temperature on sporophyte length reported by the statistical analysis, and not effects of initial size, I normalized the data to sample size. I first produced a simple regression to model the average effect of initial sporophyte length on final length (for an example, see **Figure 2.1**). I then interpreted deviations from the fitted mean response (i.e. the model residuals) as effects of the applied temperature treatments (Shama, 2017).

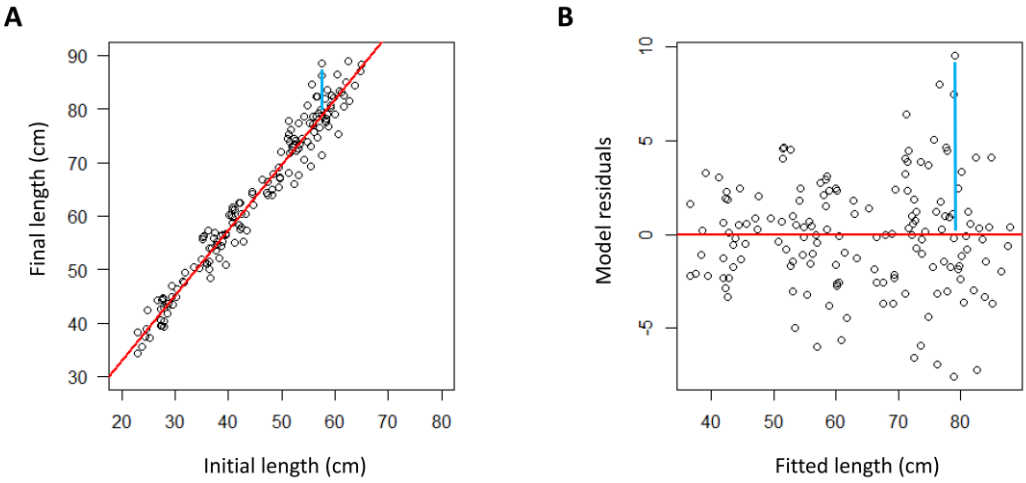

Figure 2.1 Example for producing model residuals to compare growth in experimental treatments among differentially sized samples. **(A)** Simple linear model (regression) of length at the end of the experiment as a function of initial length before the experimental treatments. Points are measurements of individual samples, the red line shows the modelled mean response, the blue line marks the residual (vertical distance) of one point to the mean response. **(B)** Deviation (residuals) of individual measurements (exemplified by blue line as in (A)) from the modelled mean response (red line) over the range of fitted values for final length (x-axis). As the fitted length is only based on the initial size, residuals can be interpreted as experimental treatment effects.

Growth is closely interlinked with the assimilation and storage of carbon (Gómez and Huovinen, 2012), and with the availability of nutrients, whereas nitrogen is most often the limiting nutrient for seaweed growth in natural systems (Roleda and Hurd, 2019). To assess the biochemical composition of experimental kelp samples in **Publication I** and **Publication II**, two main methods were applied in this study. For the analysis of elemental carbon (C) and nitrogen (N) contents, 2–3 mg ground,

lyophilized tissue per sample was packed into tin cartridges, compressed, and combusted at 1000°C in an elemental analyzer (EURO EA, HEKAtech GmbH, Wegberg, Germany) with acetanilide as standard (Verardo et al., 1990). The ratio of C : N can then be calculated to infer nutrient sufficiency or limitation (Atkinson and Smith, 1983; Duarte, 1992). In addition, the sugar alcohol mannitol is of interest as it is a major photosynthetic product (Gómez and Huovinen, 2012), but also functions as a compatible solute in response to osmotic stress (Karsten, 2012). Following Karsten et al. (1991), I extracted mannitol from ground, lyophilized samples in 70% ethanol for 4 h. The supernatant was collected and evaporated, and the remaining pellet was resuspended in water and analysed via high-performance liquid chromatography (HPLC; 1200 Series, Agilent Technologies).

Carbon assimilation depends on photosynthesis, which is influenced by temperature in mediating pigment, enzyme or photosystem contents (Gerard and Du Bois, 1988; Davison et al., 1991; Machalek et al., 1996; Li et al., 2019). Fluorescence of chlorophyll *a* can be used to assess the optimum quantum yield F_v/F_m (i.e. the photosynthetic efficiency) of photosystem II (PS II). Chlorophyll fluorescence of dark-acclimated samples was measured with pulse amplitude modulated (PAM) fluorometres. In this thesis, two devices were used. In **Publication I** and **Publication II**, I used a conventional PAM-2100 (Walz, Effeltrich, Germany) which can detect fluorescence in a small thallus area determined by the size of an optical fibre which is placed on the sample (here, the meristematic region of sporophytes). In **Publication III**, I used an Imaging PAM (Walz, Effeltrich, Germany) which can detect fluorescence of larger areas by use of a camera instead of an optical fibre. This allowed to record responses of entire juvenile sporophytes, whereas the area of interest was still the meristematic region. As both devices apply different technology to investigate the same parameters, results are not comparable among devices. For instance, F_v/F_m is generally reported lower in the Imaging PAM than in a conventional PAM-2100 even in photosynthetic tissue of single-cell thickness (Nielsen and Nielsen, 2008). With the PAM-2100, I additionally recorded effective quantum yield of light-acclimated samples over a range of 0–511 µmol photons m^{-2} s^{-1} in rapid light curves (RLC). Based on effective quantum yield over the irradiance gradient, relative electron transport rates (rETR) in photosystem II were calculated (Maxwell and Johnson, 2000; Hanelt, 2018). rETR vs. irradiance curves were then fit following the model of Jassby and Platt (1976) to calculate maximum relative electron transport rate $rETR_{max}$, saturation irradiance I_k, and photosynthetic efficiency α of each curve. The lack of steady-state responses due to the short illumination periods in the PAM approach used here results in approximations of $rETR_{max}$, I_k and α. These approximations may be used to describe relative changes between treatments (Enríquez and Borowitzka, 2010), but are not equivalent to measures of photosynthesis via photosynthetic gas exchange (Maxwell and Johnson, 2000). I further used the data obtained via RLC to investigate non-photochemical energy dissipation from PS II. Maximum nonphotochemical quenching NPQ_{max}, the saturation irradiance E_{50}, and the sigmoidicity coefficient *n* were calculated following Serôdio and Lavaud (2011).

Finally, I investigated chlorophyll and xanthophyll pigment contents of meristematic material in **Publication I**. Increased xanthophyll contents may indicate a photoprotective acclimation reaction (Pfündel and Bilger, 1994; Uhrmacher et al., 1995; Latowski et al., 2011), while the de-epoxididation ratio of the xanthophyll cycle pigments violaxanthin, antheraxanthin and zeaxanthin (in rising order of de-epoxidation) represents the current capacity to quench excessive energy from the photosystem (Pfündel and Bilger, 1994). Lyophilized samples were ground under dim light conditions, weighed to 50–80 mg, and extracted in 90% aqueous acetone in darkness for 24 h at 7°C. HPLC analysis followed the protocol and equipment described by Scheschonk et al. (2019).

2.4 Statistical analysis of physiological data

I conducted statistical analyses of all physiological parameters in the R statistical environment (R Core Team, 2019). Linear mixed effects models are a powerful tool to incorporate data structure and variance within the dataset into the statistical analysis (Zuur et al., 2009). For data collected for this thesis, I modelled response variables as a function of fixed effects (experimentally modulated factors), random effects (varying responses within the tested groups, e.g. among genotypes) and covariates (e.g. differences in response before the experimental treatment). I then investigated model fit by assessing the distribution of normalized model residuals over the fitted values, and by testing if normalized model residuals are homoscedastic and represent samples from a normal distribution. Non-normality and heteroscedasticity among normalized model residuals was accounted for by including a term describing the variance structure in the model (Zuur et al., 2009). If a good model fit is confirmed, the significance of correlation among fixed effects and response variables can be investigated with an F-test (i.e. analysis of variance, ANOVA). Additionally, testing for significance of random effects allows an assessment of differences in magnitude (random intercept) or direction (random slope) of within-group variance (e.g. among genotypes) over the fixed effects. I used this to determine genetic variation for plasticity among genotypes in **Publication II**. In repeated measures designs (**Publication I**), the correlation structure among observations of repeatedly measured subjects can be implemented in the model to allow comparisons among dependent observations (Pekár and Brabec, 2016).

2.5 Population genetics analysis

In **Publication I**, I related physiological heat responses to population genetics based on neutral microsatellite markers. Neutral microsatellites are tandem repeats of few nucleotides in the DNA sequence, which occur throughout the genome, are highly polymorphic, and are believed to be under no selection pressure (Ellegren, 2004; Vieira et al., 2016). Based on similarities or differences of

these sequences mainly in repeat numbers, genetic distance and diversity among populations can be inferred without confounding effects of selective processes (Ellegren, 2004; Vieira et al., 2016). Microsatellite analysis was performed in collaboration with Myriam Valero and Louise Fouqueau from the Station Biologique de Roscoff, France. DNA was extracted from 8–12 mg of dried tissue using the NucleoSpin 96 Plant II kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Lysis, microsatellite amplification and scoring was performed for 12 polymorphic loci following Robuchon et al. (2014b). We used twelve microsatellite markers, of which six were developed for *Laminaria digitata* by Billot et al. (1998) and six were developed for *Laminaria ochroleuca* by Coelho et al. (2014). Genetic diversity was characterized for each population as the mean number of alleles per locus (N_a), unbiased expected heterozygosity (H_e), observed heterozygosity (H_o), number of private alleles (P_a), allelic richness (AR) and the inbreeding coefficient (F_{IS}). The existence of differentiated genetic groups among *L. digitata* populations was analysed with a Bayesian clustering method (Pritchard et al., 2000) by testing a range of $1 \leq K \leq 6$ clusters (Gilbert et al., 2012). The most likely value of K was determined using Evanno ΔK in the software Structure Harvester (Evanno et al., 2005; Earl and vonHoldt, 2012).

2.6 RNA isolation and transcriptomic data analysis

In **Publication III**, I analysed transcriptomic responses to heat stress of *L. digitata* sporophytes to investigate thermal resilience and metabolic regulation. Due to the high levels of polysaccharides and phenolic compounds present in kelps, obtaining pure RNA for downstream analysis is difficult (Wang et al., 2005; Pearson et al., 2006; Heinrich, 2018). For extraction of high-quality RNA, I followed the extraction protocol originally published by Heinrich et al. (2012) and explained in detail by Heinrich (2018). The protocol combines a cetyltrimethylammonium bromide (CTAB) extraction and chloroform purification with the subsequent use of a commercial RNA extraction kit (RNeasy Plant Mini Kit, Qiagen, Hildesheim, Germany). Sporophytes were frozen in liquid nitrogen, stored at -80°C and processed within four weeks. RNA concentration and purity was inspected with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies LLC, Wilmington, USA) and RNA integrity was confirmed by capillary electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies, Santa Clara, USA). At Cologne Center for Genomics (CCG), RNA was polyA+ enriched and converted to a cDNA sequencing library using a TruSeq RNA Library Prep Kit (Illumina, San Diego, USA). The cDNA was sequenced as 150 bp paired end libraries on an Illumina HiSeq 2500. Reads were quality trimmed with Trimmomatic (Bolger et al., 2014).

The *L. digitata* transcriptome was assembled *de novo* in collaboration with Gernot Glöckner and Shivani Rana from the University of Cologne. We used the algorithm ASplice, which returns assembled contigs and normalized expression data (Sze et al., 2017). Based on these expression data,

I analysed all contigs which were at least 500 bp long for differential expression among treatments using the R package "DESeq2" (Love et al., 2014). For functional annotation of all differentially expressed genes, I used the "Trinotate" pipeline (Bryant et al., 2017). As *L. digitata* is not a model organism, the annotation of genes relies on the identification of orthologues in other, closely related species. Therefore, I used various databases to identify the highest coverage of functional annotation with reference to other brown algae (mainly the model brown alga *Ectocarpus siliculosus*; Peters et al., 2004; Cock et al., 2010) or diatoms. Uniref90 provided the fastest and most complete annotation, while still more than 80% of the investigated contigs remained unidentified. That the majority of differentially expressed genes cannot be identified is a common problem in transcriptomic studies of kelps (Heinrich et al., 2012; Monteiro et al., 2019b), and warrants careful interpretation of the data. Following functional annotation, I manually inspected gene functions of 151 identified and differentially expressed genes and related them to relevant pathways of heat stress responses.

3 Publication I

Daniel Liesner, Louise Fouqueau, Myriam Valero, Michael Y. Roleda, Gareth A. Pearson, Kai Bischof, Klaus Valentin, Inka Bartsch

Heat stress responses and population genetics of the kelp *Laminaria digitata* (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations

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ORIGINAL RESEARCH

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Heat stress responses and population genetics of the kelp *Laminaria digitata* (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations

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Abstract

To understand the thermal plasticity of a coastal foundation species across its latitudinal distribution, we assess physiological responses to high temperature stress in the kelp *Laminaria digitata* in combination with population genetic characteristics and relate heat resilience to genetic features and phylogeography. We hypothesize that populations from Arctic and cold-temperate locations are less heat resilient than populations from warm distributional edges. Using meristems of natural *L. digitata* populations from six locations ranging between Kongsfjorden, Spitsbergen (79°N), and Quiberon, France (47°N), we performed a common-garden heat stress experiment applying 15°C to 23°C over eight days. We assessed growth, photosynthetic quantum yield, carbon and nitrogen storage, and xanthophyll pigment contents as response traits. Population connectivity and genetic diversity were analyzed with microsatellite markers. Results from the heat stress experiment suggest that the upper temperature limit of *L. digitata* is nearly identical across its distribution range, but subtle differences in growth and stress responses were revealed for three populations from the species' ecological range margins. Two populations at the species' warm distribution limit showed higher temperature tolerance compared to other populations in growth at 19°C and recovery from 21°C (Quiberon, France), and photosynthetic quantum yield and xanthophyll pigment responses at 23°C (Helgoland, Germany). In *L. digitata* from the northernmost population (Spitsbergen, Norway), quantum yield indicated the highest heat sensitivity. Microsatellite genotyping revealed all sampled populations to be genetically distinct, with a strong hierarchical structure between southern and northern clades. Genetic diversity was lowest in the isolated population of the North Sea island of Helgoland and highest in Roscoff in the English Channel. All together, these results support the hypothesis of moderate local differentiation across *L. digitata's* European distribution, whereas effects are likely too weak to ameliorate the species' capacity to withstand ocean warming and marine heatwaves at the southern range edge.

KEYWORDS

growth rate, local adaptation, marine forest, marine heatwave, microsatellite, physiology

1 | INTRODUCTION

Temperature is one of the main drivers determining latitudinal species distributions on the global scale (Jeffree & Jeffree, 1994; Lüning, 1990; Stuart-Smith, Edgar, & Bates, 2017). For sedentary organisms, the thermal limits of the realized niche are broadly described by mean summer and winter isotherms (van den Hoek, 1982; Jeffree & Jeffree, 1994; Stuart-Smith et al., 2017), between which a species can complete its life cycle, while single extreme temperature events can further alter local species abundances especially at the range edges (Ruthrof et al., 2018; Smale, Wernberg, & Vanderklift, 2017; Straub et al., 2019). As a result of climate change, isotherms in the northern hemisphere have been shifting predominantly poleward since 1960 (Burrows et al., 2011), with consequent phenological and distributional changes in many taxa (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Poloczanska et al., 2013).

Predictions of species distributions during climate change are often based on niche models, which assume that all individuals within a species respond uniformly (King, McKeown, Smale, & Moore, 2018; Müller, Laepple, Bartsch, & Wiencke, 2009; Reed, Schindler, & Waples, 2011). Consequently, trait variability needs to be integrated into estimates of future range shifts (Bennett, Duarte, Marbà, & Wernberg, 2019; Cacciapaglia & van Woesik, 2018; Chardon, Pironon, Peterson, & Doak, 2020), especially as recent evidence suggests a central role of plasticity and local adaptation in species' responses to climate change (Atkins & Travis, 2010; Liesner, Shama, Diehl, Valentin, & Bartsch, 2020; Valladares et al., 2014).

Along cold-temperate to polar rocky shores, kelps (large brown algae in the order Laminariales) provide important ecosystem services as foundation species of marine forests (Steneck et al., 2002; Teagle, Hawkins, Moore, & Smale, 2017; Wernberg & Filbee-Dexter, 2019). Their coastal habitats are highly affected not only by gradual global warming, but also further by the accompanying changing onset of the warm season (Lima & Wethey, 2012) as well as the frequency and magnitude of extreme temperature events such as marine heatwaves (MHW; Hobday et al., 2016; Oliver et al., 2018). Poleward range shifts have already been documented for various kelp and fucoid seaweeds, which were attributed to global warming (Lima, Ribeiro, Queiroz, Hawkins, & Santos, 2007; Nicastro et al., 2013; Smale, Wernberg, Yunnie, & Vance, 2015).

Further range shifts are predicted for many species, including the North Atlantic kelp *Laminaria digitata* (Hudson) J.V. Lamouroux (Assis, Araújo, & Serrão, 2018; Raybaud et al., 2013). At high latitudes, *L. digitata* occurs on Spitsbergen and Greenland, while its southern distribution limit along the European coastline is in Brittany, France (Lüning, 1990). It thereby occurs between the 0°C winter and 18°C summer sea-surface isotherm (Müller et al., 2009) indicating its wide temperature performance range as an Arctic to cold-temperate species (sensu Lüning, 1990). Comparative laboratory studies described an upper survival temperature of western and eastern Atlantic juvenile *L. digitata* sporophytes of 23°C over seven days (Bolton & Lüning, 1982) and of 21°C over 14 days (tom Dieck, 1992), indicating high stability of thermal characteristics across regions. However, these investigations only compared single unialgal strains, which may not represent the entire species. Investigations on wild *L. digitata* sporophytes from Nova Scotia show mortality within one week at 21°C and tissue damage at 18°C (Simonson, Scheibling, & Metaxas, 2015). In South West England *L. digitata*, stress signals and reduced growth were evident after 16 days at 18°C (Hargrave, Foggo, Pessarrodona, & Smale, 2017).

L. digitata is a relatively young species, which probably originated from a Pacific ancestor crossing the Arctic toward the Atlantic ca. 5.3 million years ago (Lüning & tom Dieck, 1990; Rothman, Mattio, Anderson, & Bolton, 2017; Starko et al., 2019). Therefore, *L. digitata* was likely present in the Atlantic over multiple glacial cycles during the Quaternary (Assis et al., 2018), including the most recent Last Glacial Maximum 20,000 years ago (LGM; Clark et al., 2009). Recently, it has been proposed that *L. digitata* persisted during the LGM in only two disjoint refugia in the Northeast Atlantic, one located in the Armorican/Celtic Sea and one further north in the region of Ireland and Scotland (Neiva et al., 2020). Such a northern refugium for *L. digitata* was also suggested by King et al. (2020). Therefore, not only might the current climate since the LGM have affected thermal plasticity of *L. digitata* populations, but also the repeated retreat into glacial refugia and subsequent recolonization of the Northern Atlantic might have modulated genetic diversity and structure over several glacial cycles (Hewitt, 2004; Maggs et al., 2008). This possibly facilitated phenotypic divergence along what is presently a widespread latitudinal distribution gradient.

Local adaptation can occur along environmental gradients or in populations under unique selection pressures and affects response traits to increase the fitness of individuals in their specific environment (Kawecki & Ebert, 2004). For populations at their ecological range margins (i.e., marginal populations sensu Soulé, 1973), the unfavorable local environment can result in smaller population size and low genetic diversity (Eckert, Samis, & Lougheed, 2008; Hampe & Petit, 2005; Kawecki, 2000). Therefore, genetic drift may impair natural selection leading to maladaptation in marginal populations (Eckert et al., 2008; Pearson, Lago-Leston, & Mota, 2009). Conversely, a highly selective environment at a species' range margin might eventually facilitate local adaptation in these unique populations (reviewed by Hardie & Hutchings, 2010) and even increase their performance following climate change (Halbritter, Billeter, Edwards, & Alexander, 2015).

Meanwhile, there is much evidence for intraspecific variation among populations of seaweeds and seagrass (reviewed by King, McKeown, et al., 2018). Local adaptation might be common in kelps and seaweed populations generally, due to their low dispersal

capacity and strong spatial structuring (King, McKeown, et al., 2018; Miller et al., 2019). Studies on local adaptation in *L. digitata* suggest that differentiation between populations could have occurred due to their geographic position (range central and marginal as well as southern and northern). King et al. (2019) investigated the expression of genes coding for heat shock proteins (HSP) in response to an hour-long heat shock in *L. digitata* from Scotland (range center) and Southern England (trailing edge). Maximum HSP response was present at 4–8°C higher temperatures in the southern populations in this short-term study, despite comparably low genetic diversity (King et al., 2020). The reduced genetic diversity and altered reproductive strategy in a southern marginal population in Brittany, France, also suggests that local differentiation has taken place (Oppliger et al., 2014; Valero et al., 2011). Overall, research on integrative responses such as growth is lacking when assessing the intraspecific thermal variation of *L. digitata*. Additionally, few studies on thermal responses of kelps incorporate physiology and population genetics over large geographic scales, although they may help to better predict climate change effects (Nepper-Davidsen, Andersen, & Pedersen, 2019).

The main objective of this study was thus to assess differentiation in heat stress responses among populations of *Laminaria digitata* present along the entire Northeast Atlantic distribution zone through a mechanistic, common-garden experiment. We hypothesized that an increasing thermal selection pressure toward the southern distribution limit increased heat resilience of sporophytes from southern *L. digitata* populations. Because of high similarities of thermal characteristics across regions reported in previous comparative studies (Bolton & Lüning, 1982; tom Dieck, 1992), we expected local differentiation in response to heat to be of small extent and to occur mainly toward the upper temperature limit (see also King et al., 2019). We further expected phenotypic differentiation to occur more prominently in populations experiencing low amounts of gene flow, while we expected low genetic diversity to be associated with reduced heat resilience as a result of genetic drift and possible maladaptation, which we investigated by the use of neutral microsatellite markers.

2 | MATERIAL AND METHODS

2.1 | Sample collection and preparation

We collected 30–35 fertile *L. digitata* sporophytes (Figure 1a) from the low intertidal zone, ensuring a distance of >1 m between samples (for the samples collected by diving in Spitsbergen, this was not guaranteed), in each of the following locations during summer (Figure 1b): Stuphallet, Kongsfjorden, Spitsbergen, Norway (SPT; 78.975 N, 11.633 E; 16 July 2019; approximate SST at time of sampling: 6.5°C); north of Tromsø, Norway (TRO; 69.790 N, 19.054 E; 14 August 2018; 8.5°C); Bodø, Norway (BOD; 67.284 N, 14.383 E; 12 June 2018; 9°C); Helgoland, Germany (HLG; 54.178 N, 7.893 E; 13 August 2018; 18°C); Roscoff, France (ROS; 48.727 N, 4.005 W; 11 September 2018; 16.5°C); and Quiberon, France (QUI; 47.470 N, 3.091 W; 10 September 2018; 16°C). Sampling in Norway and France and handling of data was conducted in accordance with the French legislation on the Access to Genetic Resources and Benefit-Sharing. Maps (Figure 1b) were generated using a European Environment Agency coastline shapefile (European Environment Agency, 2019) and QGIS 3.8.2-Zanzibar software (QGIS Development Team, 2019).

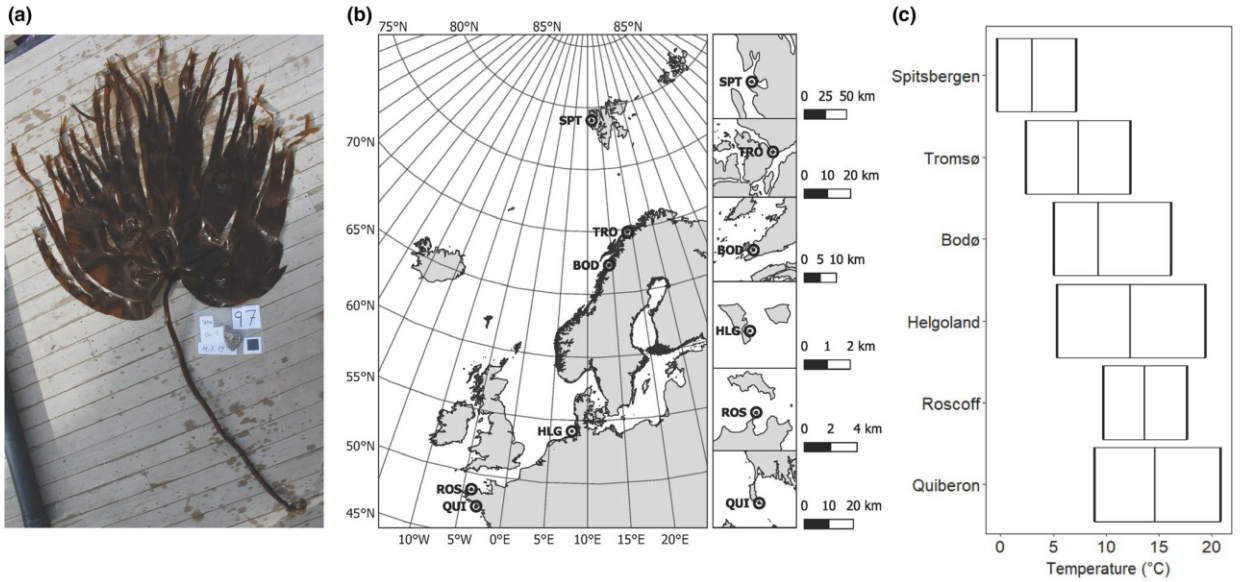

FIGURE 1 (a) Seven-year-old *Laminaria digitata* sporophyte from Spitsbergen, July 2019. The black reference square measures 5 × 5 cm. (b) Sampling locations of the *L. digitata* populations used in this study and (c) temperature amplitudes in 2018 marking minimum, mean, and maximum temperatures based on satellite-obtained mean daily sea-surface temperature datasets (E.U. Copernicus Marine Service, 2019). Abbreviations: BOD, Bodø; HLG, Helgoland; QUI, Quiberon; ROS, Roscoff; SPT, Spitsbergen; TRO, Tromsø

To represent the current temperature ranges experienced by the sampled sporophytes, satellite-obtained daily mean sea-surface temperature data (Figure 1c) with a resolution of 0.05° × 0.05° were generated representatively for 2018 using E.U. Copernicus Marine Service Information (E.U. Copernicus Marine Service, 2019).

Entire sporophytes were stored in ambient seawater for up to two days before processing. At the sampling locations, clean material from the meristematic region was preserved in silica gel for microsatellite genotyping. For the heat stress experiment, six disks (Ø 20 mm) were cut from the meristematic region of each sporophyte (i.e., 180 disks per population) in a distance of 5–10 cm from the stipe-blade transition zone. Disks were stored moist in cool boxes (<15°C) and transported to the laboratory within 30 hr. All experiments were performed at the Alfred Wegener Institute in Bremerhaven, Germany.

2.2 | Heat stress experiment

2.2.1 | Experimental design

We designed the experiment (Figure 2) as a mechanistic short-term exposure to heat stress around the upper survival temperature of *L. digitata* sporophytes (21°C for a two week exposure; tom Dieck, 1992). A temperature of 19°C was considered to be a sublethal treatment for all populations, 21°C a threshold treatment (lethal over a longer exposure time; tom Dieck, 1992; Wilson, Kay, Schmidt, & Lotze, 2015), and 23°C a critical stress treatment (Bolton & Lüning, 1982), which also surpassed mean daily maximum temperatures of all sampled populations in 2018 (Figure 1c). We exposed all samples to the same temperatures, irrespective of the ecological significance for local populations, to investigate the thermal plasticity and potential of *L. digitata* across its entire distribution range. The heat stress experiment was conducted in independent runs in common-garden conditions with material from Spitsbergen, Tromsø, Helgoland, Roscoff, and Quiberon. Due to logistic constraints, Bodø had to be excluded, and Spitsbergen material was only tested for growth and fluorescence characteristics and not for biochemistry and pigments.

For each population, five replicate pools each contained all meristem disks of six distinct sporophytes to prevent pseudoreplication. Meristem disks were transferred into sterile 5 L glass bottles filled with modified half-strength Provasoli-enriched natural seawater (PES; Provasoli, 1968; modifications: HEPES buffer instead of TRIS, double concentration of Na_2glycerophosphate; iodine enrichment following Tatewaki, 1966), which was exchanged every 3–4 days. Irradiance ranged between 30 and 35 μmol photons m^{-2} s^{-1} at the bottom of the beakers in a 16:8-hr light:dark (L:D) cycle (ProfiLux 3 with LED Mitras daylight 150, GHL Advanced Technology, Kaiserslautern, Germany). Beakers were aerated gently to ensure motion of disks and even light and nutrient availability.

To allow recovery from sampling stress, disks were cultivated at 10°C for two (Tromsø) or nine days (Spitsbergen due to logistic issues), or at 15°C for four (Roscoff, Quiberon) or three days (Helgoland) before the acclimation phase of the experiment. From each replicate pool, eight disks were then randomly assigned to one replicate 2 L glass beaker in each of the four temperature treatment groups (15, 19, 21, 23°C, n = 5). Six disks per replicate were marked by punching a small hole on the outer rim with a Pasteur pipette to be frozen for biochemical and pigment analysis during the experiment. The two unmarked disks were used for growth and fluorometric measurements over the course of the experiment.

At the beginning of the experiment, disks were acclimated at 15°C for five days to obtain a similar metabolic state (day −5 to day 0; Figure 2). Although the northern populations Spitsbergen and Tromsø do not usually experience temperatures this high (Figure 1c), 15°C is a temperature within the growth optimum of *L. digitata* (Bolton & Lüning, 1982; tom Dieck, 1992), which is considered to be stable (Wiencke, Bartsch, Bischoff, Peters, & Breeman, 1994), even for the Spitsbergen population (Franke, 2019). Starting the heat stress treatment on day 0, temperature was increased by increments of 2°C day^{-1} until the desired temperature was reached. The maximum temperature 23°C was applied for five days, while 21°C and 19°C were applied for six and seven days, respectively, according to the acclimation scheme (Figure 2). On day 8, temperature was set to 15°C for all treatment groups to initiate a recovery period of seven days. Measurements took place at the beginning of the experiment (day −5; Figure 2),

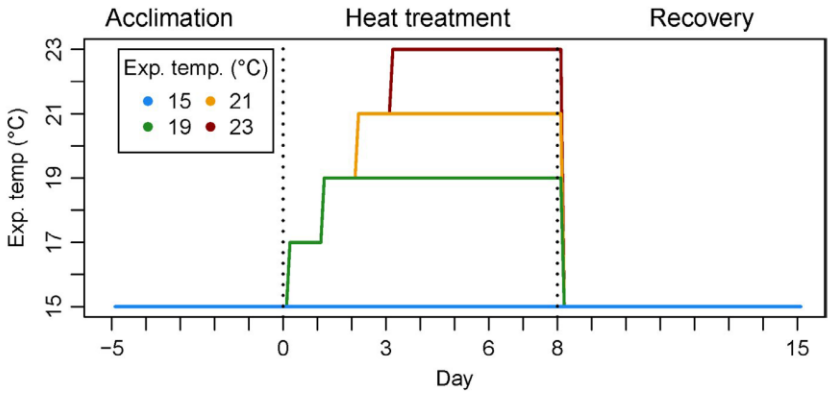

FIGURE 2 Timeline of the heat stress experiment of *Laminaria digitata*. Dotted lines separate experimental phases of acclimation at 15°C (days −5–0), heat treatment (days 0–8), and recovery at 15°C (days 8–15). Growth and F_v/F_m were measured on days −5, 0, 3, 6, 8, and 15. On days 0 and 8, rapid light curves were performed and samples were frozen for biochemical and pigment analyses

the beginning of the heat treatment (day 0), before applying the maximum temperature 23°C (day 3), in the middle of the heat treatment (day 6), at the end of the heat treatment (day 8), and after the recovery period (day 15).

2.2.2 | Relative growth rates

Two disks per replicate were repeatedly measured for growth over the course of the experiment (n = 5). Disks were blotted dry and weighed for growth analyses. Relative growth rates (RGR) were calculated as

$$\mathrm{RGR}\left(\mathrm{g\,g^{-1}\,day^{-1}}\right)=\frac{\ln x_2-\ln x_1}{t_2-t_1}$$

where x_1 = weight (g) at time 1, x_2 = weight at time 2, t_1 = time 1 in days, and t_2 = time 2 in days.

2.2.3 | PAM Fluorometry

Fluorescence parameters were assessed to estimate photoacclimation reactions in response to temperature (Davison, Greene, & Podolak, 1991; Machalek, Davison, & Falkowski, 1996) and were all conducted using a PAM-2100 chlorophyll fluorometer (Walz, Effeltrich, Germany). Maximum quantum yield of photosystem II (F_v/F_m) was repeatedly measured in two disks per replicate over the course of the experiment following 5 min dark acclimation (n = 5). Before and after the heat treatment (day 0 and day 8), rapid light curves (RLC) were conducted after F_v/F_m measurements on one disk (n = 3). RLC irradiance steps ranged from 0 to 511 μmol photons m^{-2} s^{-1}. Based on the photon flux density (PFD) and the effective quantum yield, relative electron transport rates (rETR) in photosystem II were calculated following Hanelt (2018) as

$$\mathrm{rETR}=\mathrm{PFD}\times\mathrm{Yield}$$

rETR was plotted against PFD, and the resulting curves were fitted following the model of Jassby and Platt (1976) to calculate the maximum relative electron transport rate $rETR_{max}$, the saturation irradiance I_k, and the photosynthetic efficiency α of each curve.

Nonphotochemical quenching was calculated following Serôdio and Lavaud (2011) as

$$\mathrm{NPQ}=\frac{F_m-Fm'}{Fm'}$$

where F_m = maximum fluorescence of a dark-adapted sample, and F_m' = maximum fluorescence of a light-adapted sample.

NPQ versus irradiance curves were fitted following the model of Serôdio and Lavaud (2011) to calculate maximum nonphotochemical quenching NPQ_{max}, the saturation irradiance E_{50}, and the sigmoidicity coefficient n.

2.2.4 | Biochemistry

Biochemical and pigment analyses were conducted with material from Tromsø, Helgoland, Roscoff, and Quiberon. We assessed the early photosynthetic product mannitol, which is accumulated during summer (Schiener, Black, Stanley, & Green, 2015), and elemental carbon and nitrogen to estimate carbon assimilation and nutrient storage in response to temperature. In wild sporophytes, assimilated mannitol is metabolized into the long-term storage polysaccharide laminarin and translocated into the distal thallus (Gómez & Huovinen, 2012; Yamaguchi, Ikawa, & Nisizawa, 1966). As the meristematic region only contains minimal amounts of laminarin in wild sporophytes (Black, 1954), and as maximum laminarin contents occur with a seasonal delay of 1–2 months in late autumn (Haug & Jensen, 1954; Schiener et al., 2015), we did not assess laminarin storage in our short-term experiment on isolated meristematic disks.

Before the start and at the end of the heat treatment (day 0 and day 8), three disks per replicate beaker (n = 5) were frozen in liquid nitrogen for biochemical and pigment analysis and stored at −80°C. For mannitol, carbon, and nitrogen analyses, samples were lyophilized and ground to a fine powder. For the analysis of carbon and nitrogen contents, 2–3 mg ground tissue per sample was packed into tin cartridges, compressed, and combusted at 1,000°C in an elemental analyzer (EURO EA, HEKAtech GmbH) with acetanilide as standard. Mannitol was extracted in 70% ethanol from three technical replicates of each experimental sample (Karsten, Thomas, Weykam, Daniel, & Kirst, 1991). Analysis was performed in an HPLC Agilent Technologies system (1200 Series) with an Aminex Fast Carbohydrate Analysis Column HPAP (100 × 7.8 mm, 9 μm, Bio-Rad, Munich, Germany) protected by a guard cartridge (Phenomenex, Carbo-Pb-2 + 4 × 3.00 mm I.D., Aschaffenburg, Germany).

2.2.5 | Pigments

We assessed chlorophyll and xanthophyll pigments in response to heat stress as a further indicator of photoprotection (Bischof & Rautenberger, 2012; Uhrmacher, Hanelt, & Nultsch, 1995). Pigment samples were lyophilized separately from biochemical samples (n = 5). They were ground under dim light conditions, weighed to 50–80 mg, and extracted in 90% aqueous acetone in darkness for 24 hr at 7°C. HPLC analysis followed the protocol and equipment described by Scheschonk et al. (2019), using a LaChromElite system (L-2200 autosampler with Cooling Unit; DAD detector L-2450; VWR-Hitachi International) with a Spherisorb ODS-2 column (25 cm × 4.6 mm, 5 μm particle size, Waters, Milford, USA) protected by a guard cartridge (LiChrospher 100-RP-18; Merck). The elution gradient was applied according to Wright et al. (1991). We used standards of chlorophyll *a* and *c*, fucoxanthin, β-carotene, violaxanthin, antheraxanthin, and zeaxanthin (DHI lab products, Hørsholm, Denmark). To assess parameters of photoprotection as a stress response, we calculated the mass ratio of xanthophyll pigments violaxanthin (V),

antheraxanthin (A), and zeaxanthin (Z) per chlorophyll *a* (Chl *a*) following Bollen, Pilditch, Battershill, and Bischof (2016) as.

$$\text{VAZ:Chl } a \text{ ratio } \left(\text{mg mg}^{-1}\text{Chl } a\right) = \frac{V+A+Z}{\text{Chl } a}$$

and de-epoxidation ratio of xanthophyll cycle pigments following Colombo-Pallotta, García-Mendoza, and Ladah (2006) as.

$$\text{De}-\text{epoxidation ratio} = \frac{Z+0.5A}{V+A+Z}$$

2.2.6 | Statistical analyses of physiological parameters

As we measured two disks per replicate, we calculated growth rates and F_v/F_m from mean values per replicate. One disk was removed from the Spitsbergen 23°C treatment due to bleaching during the heating ramp. Despite identification efforts in the field, almost none of the microsatellite markers amplified in two samples from Spitsbergen (see also 2.3.2). This led to the assumption that the two samples were of *Hedophyllum nigripes* (J. Agardh) Starko, S.C.Lindstrom & Martone, which is morphologically very similar to *L. digitata* (Dankworth, Heinrich, Fredriksen, & Bartsch, 2020; Longtin & Saunders, 2015). One replicate pool probably containing meristem disks from both species was therefore removed from the experiment. Due to the mannitol extraction performed in triplicates, means of the three subsamples of each mannitol replicate were analyzed. In carbon and nitrogen analyses, four data points were deleted due to a measuring error on day 0. In the xanthophyll pool and de-epoxidation analyses, one outlier was deleted due to implausibly high zeaxanthin contents about four times higher than the next highest value.

All analyses of the heat stress experiment were performed in the R statistical environment version 3.6.0 (R Core Team, 2019). We fitted generalized least squares models for all parameters and tested for significance using analyses of variance (ANOVA). All models were fitted using the "gls" function from the R package "nlme" (Pinheiro, Bates, DebRoy, & Sarkar, 2019) with weights arguments to counteract heterogeneity of variance of normalized model residuals (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Normalized model residuals were assessed with Shapiro–Wilk normality tests and Levene's tests for homogeneity of variance. For repeated measures analyses of variance (RM ANOVA) of growth rates and F_v/F_m, temperature, population, and time were modeled as interactive fixed effects and a compound symmetry correlation structure was incorporated using a time covariate and replicate as grouping factor (Pekár & Brabec, 2016; Zuur et al., 2009). Analyses of variance were then performed on the models with the "anova" function to assess the effects of the fixed effects temperature, population and exposure time, and their interactions. For all biochemical, pigment, and fluorometric analyses, initial contents at day 0 were incorporated in the models as covariates to account for baseline differences, and temperature and population were modeled as fixed effects. Analyses of variance were performed to assess the effects of the initial value covariate and the fixed effects temperature and population, and their interaction. Pairwise comparisons were performed using the R package "emmeans" (Lenth, 2019) and using the "Satterthwaite" mode for calculation of degrees of freedom and Tukey adjustment of *p*-values for multiple comparisons between independent groups. For pairwise comparisons in the repeated measures analyses (growth and F_v/F_m), the "df.error" mode for calculation of degrees of freedom was applied. Because of the repeated measures design and because the "df.error" mode overestimates the degrees of freedom (Lenth, 2019), *p*-values were adjusted by means of the conservative Bonferroni correction for multiple testing to reduce the probability of type I errors. Correlation analyses (Kendall's rank correlation) were conducted between all parameters measured after the heat treatment (relative growth rates calculated between day 0 and day 8) using the "cor.test" function from the default R package "stats" (R Core Team, 2019).

2.3 | Microsatellite genotyping

2.3.1 | DNA extraction

DNA was extracted from 8–12 mg of dried tissue using the NucleoSpin 96 Plant II kit (Macherey-Nagel GmbH & Co. KG) following the manufacturer's instructions. The lysis, microsatellite amplification and scoring was performed for 12 polymorphic loci following Robuchon, Le Gall, Mauger, and Valero (2014). Multiplex PCRs were modified using 5X GoTaq Flexi colorless reaction buffer (Promega Corp., Madison, USA) instead of 1X and performed using a T100™ Thermal Cycler (Bio-Rad Laboratories Inc.).

2.3.2 | Microsatellite amplification, scoring, and correction

Among the markers used, six were previously developed for *Laminaria digitata* (Ld148, Ld158, Ld167, Ld371, Ld531, and Ld704; Billot et al., 1998) and six for *Laminaria ochroleuca* (Lo4-24, Lo454-17, Lo454-23, Lo454-24, Lo454-27, and Lo454-28; Coelho, Serrão, & Alberto, 2014). Alleles were sized using the SM594 size standard (Mauger, Couceiro, & Valero, 2012) and scored manually using GeneMapper 4.0 (Applied Biosystems). Individuals, for which more than one locus did not amplify, were removed from the dataset. Amplification was faulty for the population of Helgoland sampled in 2018, which could be linked to poor preservation or insufficient dehydration. Therefore, the dataset of the same population sampled at the same site in 2016 was used in the genetic analysis instead. In total, 190 individuals were initially genotyped for twelve microsatellite markers and 179 were retained.

2.3.3 | Genetic diversity

Prior to genetic analysis, the presence of null alleles was tested using the ENA method in FreeNa (Chapuis & Estoup, 2007). Single and multilocus estimates of genetic diversity were calculated for

each population as the mean number of alleles per locus (N_a), unbiased expected heterozygosity (H_e, sensu Nei, 1978), observed heterozygosity (H_o), and number of private alleles (P_a) using GenAlEx 6.5 (Peakall & Smouse, 2006). In addition, allelic richness (AR) was computed using FSTAT 2.9.3 (Goudet, 2001) for each locus using the rarefaction method. Linkage disequilibrium between pairs of loci and single estimates of deviation from random mating (F_{IS}) was calculated according to Weir and Cockerham (1984), and statistical significance was computed using FSTAT based on 7920 permutations for linkage disequilibrium and 10^4 for F_{IS}. To test the null hypothesis that populations did not differ in genetic diversity, a one-way ANOVA was performed for AR, P_a, and H_e in R (R Core Team, 2019). Pairwise differences between means were tested by Fisher Individual Tests for Differences of Means (Minitab® Statistical Software, version 19.2). The homoscedasticity of the dataset and the normality of residuals was visually checked prior to the analyses.

2.3.4 | Population structure

Population structure was investigated first by the analysis of the pairwise estimates of F_{ST} (Weir & Cockerham, 1984), and their significance were computed using FSTAT (Goudet, 2001). Second, a Bayesian clustering method as implemented in Structure 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) was used to determine the existence of differentiated genetic groups within *L. digitata* populations categorizing them into K subpopulations. A range of clusters (K) from one to six was tested with 100 iterations, a burn-in period of 100,000, and a Markov chain Monte Carlo of 500,000 (Gilbert et al., 2012). The most likely value of K was determined using Evanno ΔK (Evanno, Regnaut, & Goudet, 2005) obtained using Structure Harvester (Earl & vonHoldt, 2012). Replicates of Structure runs were combined using CLUMPP software (Jakobsson & Rosenberg, 2007). Bar plots were created with Distruct (Rosenberg, 2004).

3 | RESULTS

3.1 | Heat stress experiment

The significant main effects of independent factors are only reported in the absence of significant interactive effects. Therefore, in the presence of significant interactive effects, the simultaneous effects of two or more independent variables on a given dependent variable are given more emphasis than significant main effects.

3.1.1 | Growth

The significant population × temperature × time interaction for relative growth rates (Figure 3; Table 1) indicates that growth in the temperature treatments differed significantly between populations over exposure time. However, there were differences in general growth activity between populations already during acclimation at 15°C (Figure 3a), which persisted during the heat and recovery phases (Figure 3b,c), indicating a different physiological status among populations. This is represented by the significant main effect of population on growth rates (Figure 3; Table 1). Mean growth over all temperatures and time points was significantly lower in material from the northern populations Spitsbergen and Tromsø (by 34%–70%) than in material from the southern populations Helgoland, Roscoff, and Quiberon ((ROS = QUI) > HLG > SPT > TRO, Bonferroni-corrected pairwise comparisons, $p < .001$).

During the heat stress treatment (Figure 3b), interactive effects of temperatures and populations became evident. While temperature effect sizes were small in the northern populations, possibly because of the generally low growth activity, growth rates of Helgoland, Roscoff, and Quiberon material at 21°C and 23°C were 50%–60% lower than at 15°C. In both Helgoland and Roscoff samples, 19°C–23°C significantly reduced growth compared to the 15°C control (Bonferroni test, $p < .01$), whereas samples from Quiberon grew significantly slower only at 21°C and 23°C compared to 15°C (Bonferroni tests, $p < .001$). Quiberon was the only population where growth did not decrease significantly at 19°C neither over time nor compared to the 15°C control.

Over the recovery period at 15°C (Figure 3c), specimens from all populations showed significantly decreased growth after exposure to 23°C compared to lower temperature treatments (Bonferroni tests, $p < .05$). Spitsbergen and Tromsø essentially ceased growth (RGR < 0.001 and 0.002 g g^{-1} day^{-1}, respectively), while Helgoland, Roscoff, and Quiberon maintained slow growth (0.006, 0.004, and 0.01 g g^{-1} day^{-1}, respectively). However, during recovery after exposure to 23°C, there were no significant differences between growth rates of the different populations (Bonferroni tests, $p > .05$). Quiberon material recovered best, in that there were no significant differences between the 15 and 21°C treatments while disks in these treatments simultaneously grew significantly faster than those from the former 23°C treatment (Bonferroni tests, $p < .01$).

In the more detailed time course of growth rates (Figure A1), it became evident that all populations showed a trend of recovery from 21°C as growth rates increased between day 8 and day 15 (Figure A1), which was significant only for Quiberon (RM ANOVA; Table A1; Bonferroni test, $p < .001$) and Spitsbergen (RM ANOVA; Table A1; Bonferroni test, $p < .01$). Additionally, only Helgoland and Quiberon material slightly, but not significantly, recovered growth rates from the 23°C treatment (RM ANOVAs; Table A1; Bonferroni tests, $p > .05$). At the end of the experiment, one Spitsbergen disk had bleached in the 23°C treatment, while all other disks survived.

3.1.2 | Photoacclimative responses

Maximum quantum yield of photosystem II (F_v/F_m) in the temperature treatments differed between populations over time, which is represented by the significant population × temperature × time

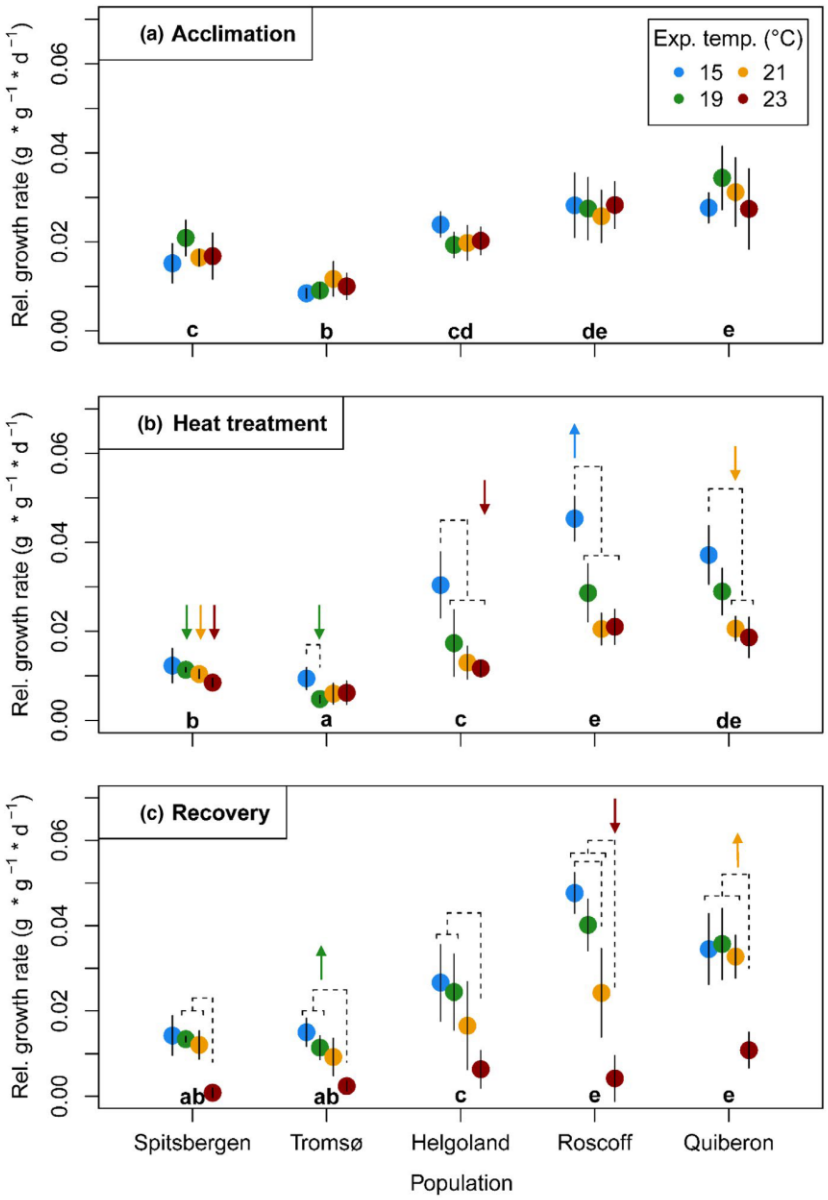

FIGURE 3 Relative growth rates of *Laminaria digitata* disks over the experimental phases of (a) acclimation at 15°C, (b) heat treatment, and (c) recovery at 15°C. Mean values ± *SD* ($n = 5$, for Spitsbergen $n = 4$). Lowercase letters indicate significant differences between all mean population responses over time (Bonferroni tests, $p < .05$). Dashed lines indicate significant differences between temperature treatments within populations (Bonferroni tests, $p < .05$). Arrows indicate significant differences between temperature treatments over time (Bonferroni tests, $p < .05$). Significance levels are given in the text

interaction (Figure 4, Table 1). After acclimation, all samples showed no signs of stress with F_v/F_m ranging between 0.7 and 0.8 (Figure 4a).

At the end of the heat treatment (Figure 4b), temperature effects on quantum yield contrasted between the two populations of Spitsbergen and Helgoland. Spitsbergen material was most susceptible to the heat treatments: At 21°C and 23°C, quantum yield was significantly lower (by 12% and 25%, respectively) than at 15°C and 19°C (Bonferroni tests, $p < .001$). Tromsø, Roscoff, and Quiberon samples responded with a significant decrease in quantum yield by 11%–13% only at 23°C (Bonferroni tests, $p < .05$). In contrast, Helgoland samples were most stress resistant and showed a general stability of quantum yield in all conditions over time. Only at 23°C, at the end of the heat treatment, there was a slight decrease in quantum yield (significantly different only to the 19°C treatment; Bonferroni test, $p < .001$), but F_v/F_m was still significantly higher (9%–28%) than in all other populations at 23°C (Bonferroni tests, $p < .01$).

At higher temporal resolution (Figure A2), a general difference between southern and northern populations became more pronounced. While the significant decrease in quantum yield at 23°C took place between day 6 and day 8 for Helgoland, Roscoff, and Quiberon (RM ANOVA; Table A1; Bonferroni tests, $p < .05$), this decrease already started between day 3 and 6 in Spitsbergen and Tromsø material (Bonferroni tests, $p < .001$). Only specimens from Spitsbergen, as the most susceptible population, significantly decreased quantum yield also at 21°C, between day 6 and day 8 (Bonferroni test, $p < .01$).

The stronger heat susceptibility of Spitsbergen material became evident also following the recovery period (Figure 4c). While all other populations recovered from 23°C, in that there were no

TABLE 1 Results of generalized least squares models to examine variability of relative growth rates (RGR) and maximum quantum yield (F_v/F_m) of *Laminaria digitata* disks in the heat stress experiment

			RGR		F_v/F_m	
Parameter	numDF	denDF	*F*-value	*p*-value	*F*-value	*p*-value
Population	4	228	283.25	**<.0001**	36.77	**<.0001**
Temperature	3	228	60.38	**<.0001**	29.06	**<.0001**
Time	2	228	54.56	**<.0001**	104.37	**<.0001**
Population × temperature	12	228	12.13	**<.0001**	5.56	**<.0001**
Population × time	8	228	7.70	**<.0001**	8.09	**<.0001**
Temperature × time	6	228	31.83	**<.0001**	32.91	**<.0001**
Population × temperature × time	24	228	3.20	**<.0001**	5.58	**<.0001**

Note: Fresh weight relative growth rates and maximum quantum yield F_v/F_m over acclimation, heat treatment, and recovery periods were tested against interactive effects of population, heat stress temperature treatment, and time. Tested values are means of 2 per replicate ($n = 5$, $n = 4$ for Spitsbergen). numDF, numerator degrees of freedom; denDF, denominator degrees of freedom. Statistically significant values are indicated in bold text.

significant differences to the 15°C control, Spitsbergen only recovered successfully from 21°C (Bonferroni tests, $p > .05$). However, F_v/F_m did not recover in Spitsbergen material following the 23°C treatment (compared to 15–19°C; Bonferroni tests, $p < .01$), indicating chronic photoinhibition and likely damage to photosystem II.

Contrary to quantum yield, the photoacclimation parameters obtained from rapid light curves at the end of the heat treatment, maximum relative electron transport rate $rETR_{max}$ (Figure A3a), saturation irradiance I_k (Figure A3b), and photosynthetic efficiency α (Figure A3c) did not show significant effects or interactions of temperature and population (Table A2). In contrast, nonphotochemical quenching (NPQ) parameters showed no significant interaction effects, but significant effects of population on maximum nonphotochemical quenching NPQ_{max} and saturation irradiance E_{50}, and of temperature on the sigmoidicity coefficient *n* (Figure A4; Table A3). Mean NPQ_{max} (Figure A4a) was 47%–56% lower in Helgoland material than in Tromsø, Roscoff, and Quiberon over all temperatures ((QUI = ROS =TRO = SPT) > (SPT = HLG); Tukey tests, $p < .05$), indicating intrinsically low nonphotochemical quenching in the Helgoland population. Mean E_{50} (Figure A4b) of Spitsbergen material was significantly lower than in Tromsø, Helgoland, and Quiberon by 29%–38% over all temperatures ((QUI = ROS =HLG = TRO) > (ROS = SPT); Tukey tests, $p < .05$), indicating an onset of NPQ already at low irradiances for Spitsbergen. The significant effect of temperature on *n* (Figure A4c) was visible as a mean downward trend of *n* by 29% between 15 and 23°C over all populations ((15°C = 19°C) > (19°C = 21 °C) > (21°C = 23°C); Tukey tests, $p < .001$), indicating a greater response of NPQ under lower irradiances at high temperatures.

3.1.3 | Biochemistry

Tissue mannitol and carbon contents were not significantly affected by interactive effects of population and temperature (Figure 5; Table 2), indicating that all populations responded uniformly to the temperature treatments in carbon storage. The significant effect of population on mannitol contents (Table 2) was due to the lowest contents in Roscoff and 80% higher contents in Tromsø material (TRO > (HLG = QUI) > ROS; Tukey tests, $p < .05$). The significant effect of temperature on mannitol (Table 2) shows that 21°C and 23°C induced significantly higher mannitol contents compared to the 15°C and 19°C treatments over all populations ((23°C = 21°C) > (19°C = 15°C); Tukey tests, $p < .05$). Carbon contents were not affected by temperature, but differed significantly only between populations (Figure 5b; Table 2). As with mannitol, Tromsø material maintained a higher carbon content, in that the means were significantly (7%–9%) higher in Tromsø and Helgoland material than in Roscoff and Quiberon material ((TRO = HLG) > (ROS = QUI); Tukey tests, $p < .001$).

Nitrogen contents were significantly affected by interactive effects of population and temperature (Figure 5c; Table 2). Only Roscoff and Quiberon samples showed a significant decrease in nitrogen contents at high temperatures (at 23°C for Roscoff, Tukey tests, $p < .05$; at 21°C and 23°C for Quiberon, Tukey tests, $p < .001$). Compared to the 15°C control, 23°C led to a reduction in nitrogen content by 20% in Roscoff and 15% in Quiberon samples. In a pattern reverse to that of nitrogen, molar C:N ratios were significantly affected by interactive effects of population and temperature (Figure 5d; Table 2). C:N ratios in the 21°C and 23°C treatments were therefore significantly higher than in the 15°C control for Roscoff and Quiberon samples (Tukey tests, $p < .05$).

The model covariate for initial values had a significant effect on all biochemical parameters taken at the end of the experiment (Table 2), in which higher initial values were correlated with higher values at the end of the heat treatment. Significant negative correlations of growth rates with mannitol (Kendall's tau = −0.5570; $p < .0001$; Table A4), carbon (Kendall's tau = −0.4218; $p < .0001$), and nitrogen contents (Kendall's tau = −0.2547, $p = .0011$) indicated growth at the expense of storage.

3.1.4 | Pigments

Chlorophyll *a* content was not significantly affected by interactive effects of population and temperature, but differed

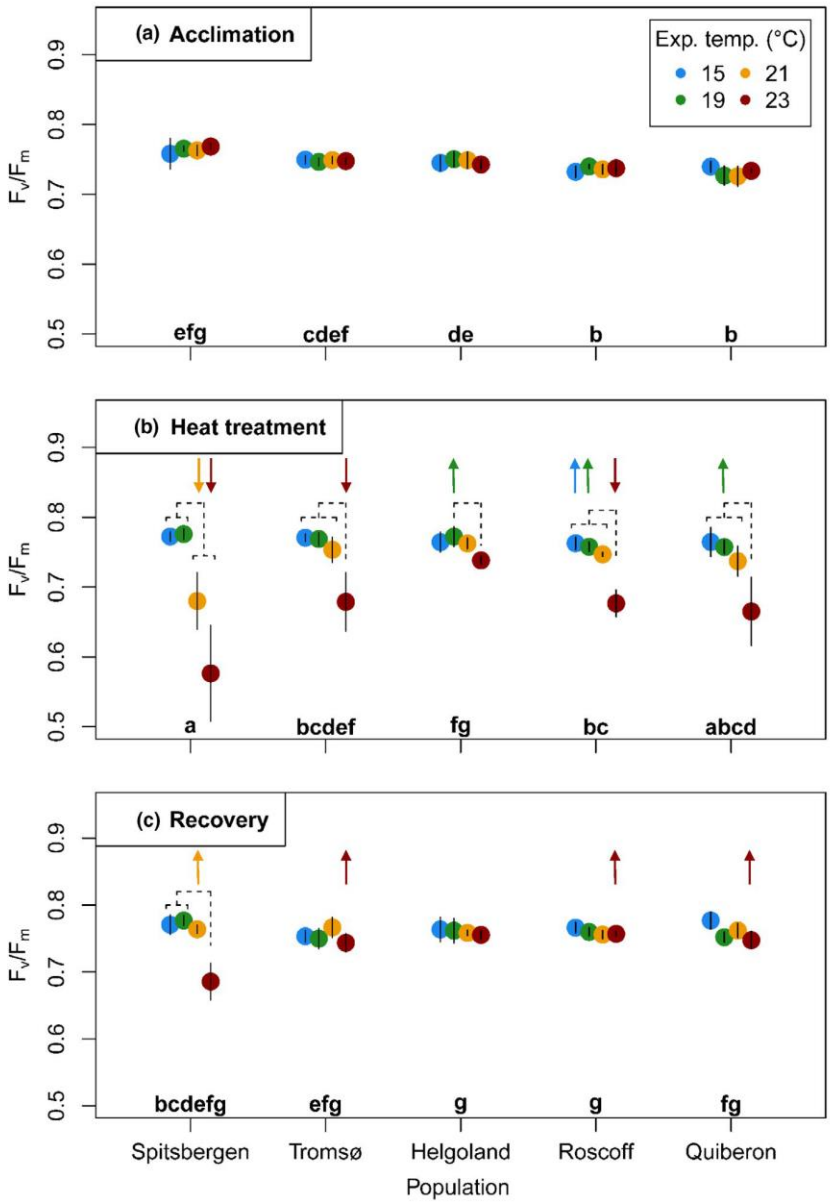

FIGURE 4 Maximum quantum yield (F_v/F_m) of *Laminaria digitata* disks after the experimental phases of (a) acclimation at 15°C, (b) heat treatment, and (c) recovery at 15°C. Mean values ± *SD* ($n = 5$, for Spitsbergen $n = 4$). Lowercase letters indicate significant differences between all mean population responses over time (Bonferroni tests, $p < .05$). Dashed lines indicate significant differences between temperature treatments within populations (Bonferroni tests, $p < .05$). Arrows indicate significant differences between temperature treatments over time (Bonferroni tests, $p < .05$). Significance levels are given in the text

significantly between populations (Figure 6a; Table 3). Mean chlorophyll *a* contents were significantly (24%–36%) lower in Tromsø samples than in Roscoff and Quiberon material ((QUI = ROS = HLG) > (HLG = TRO); Tukey tests, $p < .05$), while chlorophyll *a* content in Helgoland material did not differ significantly from the other populations.

The mass ratio of xanthophyll pigments per chlorophyll *a* (VAZ : Chl *a* ratio) was affected significantly by initial values, and interactive effects of population and temperature (Figure 6b; Table 3). Temperature had a significant, overall increasing effect on VAZ : Chl *a* ratios (23°C > 21°C > (19°C = 15°C), Tukey tests, $p < .05$), indicating accumulation of xanthophyll pigments as a photoprotective stress response toward temperature. Tromsø material significantly increased VAZ : Chl *a* ratios in the 21°C and 23°C treatments compared to the 15°C control (Tukey tests, $p < .05$) by 20% and 34%, respectively. A significant increase in VAZ : Chl *a* ratios became evident in the 23°C treatment compared to all other temperatures within the Roscoff (Tukey tests, $p < .05$) and Quiberon (Tukey tests, $p < .01$) populations. Compared to the 15°C control, 23°C led to an increase in VAZ : Chl *a* by more than 50% for both populations from Brittany, thereby presenting the strongest response in xanthophyll accumulation. In contrast, no significant differences between temperature treatments arose within the Helgoland population, further demonstrating a lack of heat stress response.

De-epoxidation ratios of xanthophyll cycle pigments were affected significantly by initial values, and interactive effects of population and temperature (Figure 6c, Table 3). The significant differences between populations in mean de-epoxidation ratios over

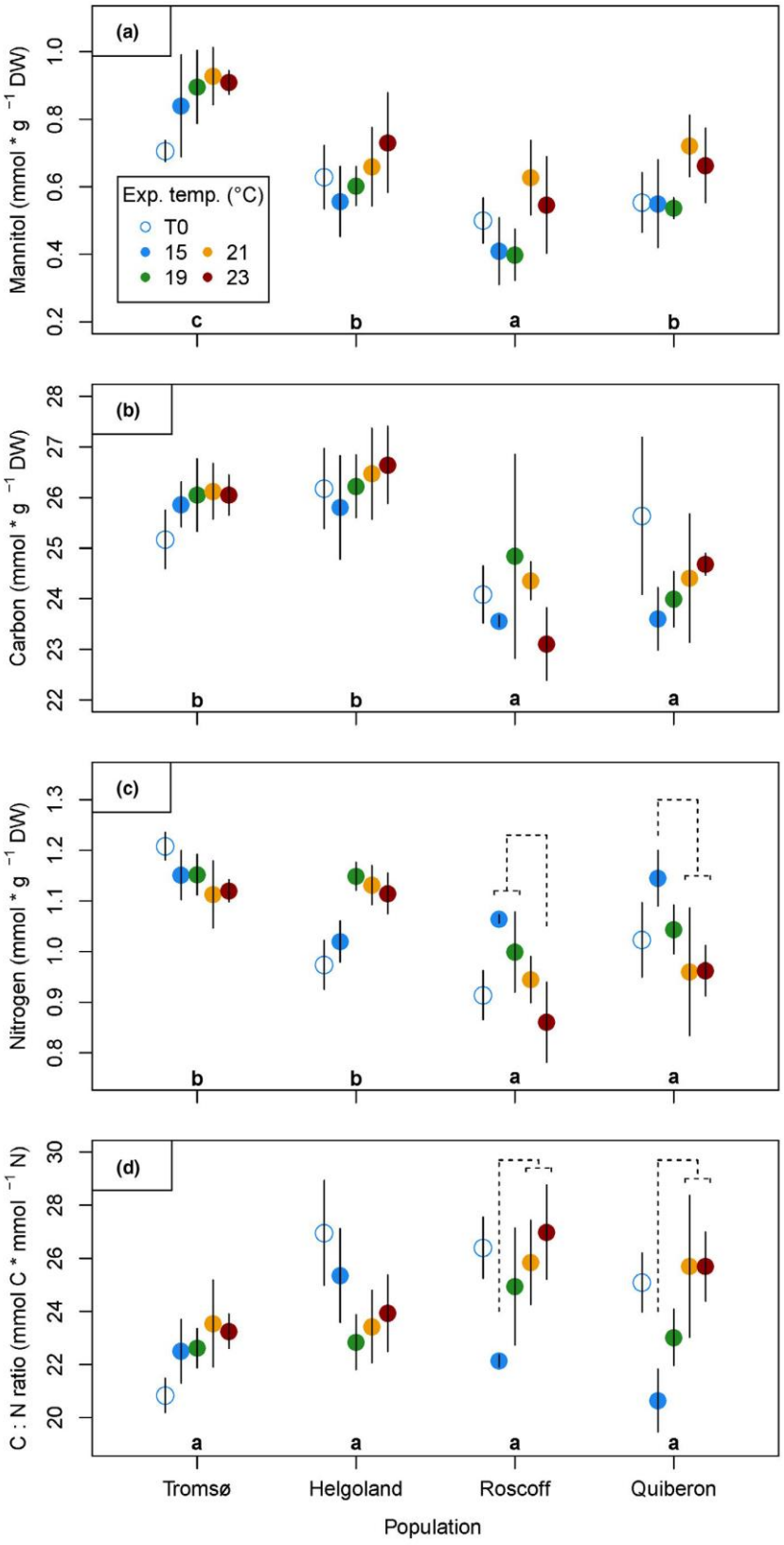

FIGURE 5 Biochemical characteristics of *Laminaria digitata* disks after acclimation at 15°C (day 0, empty circles) and after the heat treatment (day 8, colored points). (a) Mannitol contents, (b) carbon contents, (c) nitrogen contents, (d) molar C:N ratio. Mean values ± *SD* ($n = 5$, $n = 4$ for Quiberon in carbon, nitrogen, and C:N ratio), except for (a) means of mean values due to extraction in triplicates. Significant differences between mean population responses are indicated by lowercase letters (Tukey tests, $p < .05$). Significant differences between temperature treatments within populations are indicated by dashed lines (Tukey tests, $p < .05$). Significance levels are given in the text

TABLE 2 Results of generalized least squares models to examine variability of biochemical characteristics of *Laminaria digitata* disks in the heat stress experiment

			Mannitol		Carbon		Nitrogen		C:N ratio	
Parameter	numDF	denDF	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Initial values	1	63 (59)	96.04	**<.0001**	65.82	**<.0001**	49.08	**<.0001**	8.56	**.0049**
Population	3	63 (59)	19.54	**<.0001**	42.76	**<.0001**	17.48	**<.0001**	2.93	**.0410**
Temperature	3	63 (59)	9.67	**<.0001**	2.46	.0718	7.78	**.0002**	8.63	**.0001**
Population × temperature	9	63 (59)	0.92	.5133	1.90	.0688	6.18	**<.0001**	4.82	**.0001**

Note: Molar mannitol content, carbon content, nitrogen content, and C:N ratio were tested against initial values as covariate and interactive effects of population and heat stress temperature treatment. $n = 5$, $n = 4$ for Quiberon in carbon, nitrogen, and C:N ratio. numDF, numerator degrees of freedom; denDF, denominator degrees of freedom. denDF = 59 for carbon, nitrogen, and C:N ratio. Statistically significant values are indicated in bold text.

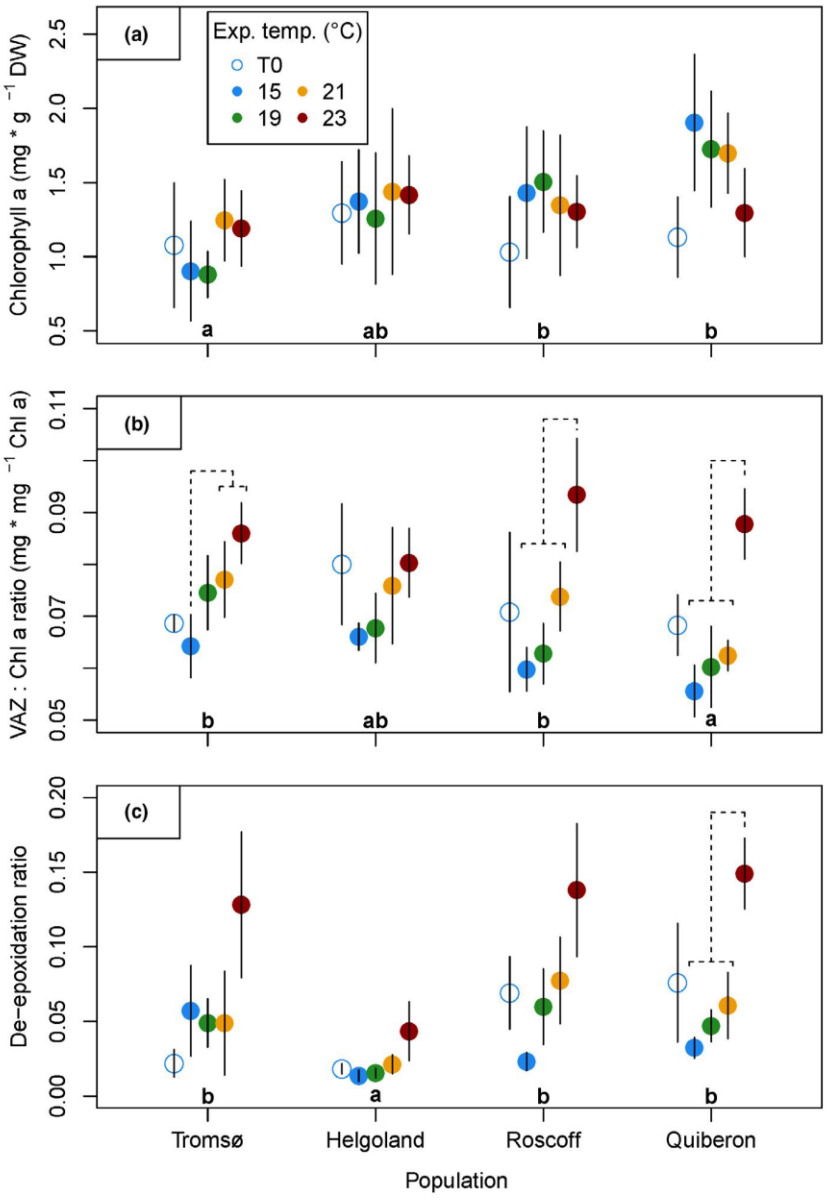

FIGURE 6 Pigment characteristics of *Laminaria digitata* disks after acclimation (day 0, empty circles) and after the heat treatment (day 8, colored points). (a) Chlorophyll *a* contents, (b) mass ratio of xanthophyll pigments per Chlorophyll *a* (VAZ : Chl *a* ratio), (c) de-epoxidation ratio of xanthophyll pigments. Mean values ± *SD* ($n = 5$, $n = 4$ for Tromsø 23°C in VAZ : Chl *a* ratio and de-epoxidation ratio). Significant differences between mean population responses are indicated by lowercase letters (Tukey tests, $p < .05$). Significant differences between temperature treatments within populations are indicated by dashed lines (Tukey tests, $p < .05$). Significance levels are given in the text

TABLE 3 Results of generalized least squares models to examine variability of pigment characteristics of *Laminaria digitata* disks in the heat stress experiment

			Chl *a*		VAZ : Chl *a* ratio		De-epoxidation ratio	
Parameter	numDF	denDF	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Initial values	1	63 (62)	1.22	.2731	22.95	**<.0001**	95.39	**<.0001**
Population	3	63 (62)	9.08	**<.0001**	3.53	**.0198**	22.96	**<.0001**
Temperature	3	63 (62)	0.53	.6653	51.39	**<.0001**	42.51	**<.0001**
Population × temperature	9	63 (62)	1.30	.2534	2.25	**.0298**	6.96	**<.0001**

Note: Chlorophyll *a* content, xanthophyll pigment (VAZ) : Chl *a* ratio, and de-epoxidation ratio were tested against initial values as covariate and interactive effects of population and heat stress temperature treatment. $n = 5$, $n = 4$ for Tromsø 23°C in VAZ : Chl *a* ratio and de-epoxidation ratio. numDF, numerator degrees of freedom; denDF, denominator degrees of freedom. denDF = 62 for VAZ : Chl *a* ratio and de-epoxidation ratio. Statistically significant values are indicated in bold text.

TABLE 4 Genetic characteristics of the *Laminaria digitata* populations used in the heat stress experiment

Population	Year	*n*	N_a	AR	P_a	H_e	H_o	F_{IS}
Spitsbergen	2019	26	3.667 ± 0.620	3.494 ± 0.427	0.250 ± 0.131	0.436 ± 0.069	0.362 ± 0.058	0.127 ± 0.054
Tromsø	2018	30	3.583 ± 0.596	3.447 ± 0.422	0.250 ± 0.131	0.363 ± 0.074	0.350 ± 0.073	0.051 ± 0.055
Bodø	2018	32	4.833 ± 1.065	4.464 ± 0.699	0.500 ± 0.195	0.444 ± 0.088	0.376 ± 0.077	0.117 ± 0.033 *
Helgoland	2016	35	2.833 ± 0.638	2.594 ± 0.422	0.083 ± 0.083	0.306 ± 0.076	0.296 ± 0.078	0.039 ± 0.032
Roscoff	2018	28	5.167 ± 1.120	4.875 ± 0.786	0.583 ± 0.229	0.480 ± 0.082	0.429 ± 0.083	0.171 ± 0.044 *
Quiberon	2018	28	4.583 ± 0.773	4.186 ± 0.511	0.333 ± 0.142	0.432 ± 0.061	0.408 ± 0.067	0.106 ± 0.035

Note: Year: year of the samples used for genetic analysis (except for Helgoland, the genotyped individuals are the same than those analyzed for the heat stress experiment); *n*, number of individuals for which at least 11 markers amplified; N_a, mean number of observed alleles; AR, allelic richness standardized for equal sample size (21 individuals); P_a, mean number of private alleles per locus; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{IS}, fixation index (inbreeding coefficient) of individuals with respect to local subpopulation. All parameters are expressed as means over all markers ± standard error. *, significant departure from random mating after correction for multiple testing ($p < .0069$, FSTAT).

all temperatures (Table 3) show that de-epoxidation ratios were significantly lower in Helgoland samples than in all other populations ((QUI = ROS = TRO) > HLG; Tukey tests, $p < .01$). This result supports low values for nonphotochemical quenching in Helgoland material (NPQ_{max}; Figure A4a). Overall, higher temperatures significantly increased de-epoxidation ratios (23°C > (21°C = 19°C) > (19°C = 15°C), Tukey tests, $p < .05$). The highest temperature of 23°C led to a mean increase in the de-epoxidation ratio by a factor of 2 in Tromsø, a factor of 3 in Helgoland, a factor of 6 in Roscoff, and a factor of 4.5 in Quiberon material compared to the respective 15°C controls. However, the only significant within-population temperature response to 23°C emerged in the Quiberon samples (Tukey tests, $p < .05$), showing the most pronounced heat response in the southernmost population.

Chlorophyll *a* content was positively correlated with growth (Kendall's tau = 0.2013; $p = .0082$; Table A4), while growth rates and VAZ : Chl *a* ratios were strongly negatively correlated (Kendall's tau = −0.2911; $p = .0001$), indicating negative effects of the heat treatments and resulting stress responses on growth. F_v/F_m after the heat treatment was strongly negatively correlated with VAZ : Chl *a* ratios (Kendalls tau = −0.2828; $p = .0002$) and to de-epoxidation ratios (Kendall's tau = −0.3954; $p < .0001$), supporting the interpretation of xanthophyll-derived parameters as photoprotective stress proxies. Additionally, de-epoxidation ratios positively correlated with maximum nonphotochemical quenching NPQ_{max} (Kendall's tau = 0.2155, $p = .0328$), further emphasizing the relation of xanthophyll pigments and photoprotection.

3.2 | Population genetics

3.2.1 | Microsatellite amplification

Null alleles were present in every population for at least two markers (Table A5). However, differences between F_{ST} values in the pairwise comparison were never greater than 10^{-3} (data not shown). Therefore, we concluded that the frequency of null alleles was negligible and our dataset was analyzed without taking into account correction for null alleles. No significant linkage disequilibrium was observed in any of the populations (Table A6). We thus considered all of the markers as independent. The number of alleles per locus ranged from 2 to 22 (Lo454-27 and Ld371, respectively).

3.2.2 | Genetic diversity

Values of genetic diversity averaged over the 12 loci are provided in Table 4 for each population (for details of genetic diversity estimates

locus by locus see Table A7). Most quantities varied by a factor of 1.5 among populations; the lowest genetic diversity was always observed in Helgoland and the highest in Roscoff. Variation was the highest for the mean number of private alleles (P_a) which ranged from 0.083 to 0.583. The differences between populations were not significant when each parameter was tested independently (one-way ANOVA, data not shown). However, a Fisher test of pairwise differences between means revealed that AR and P_a were significantly lower in Helgoland compared to Roscoff (data not shown). In addition, three of the twelve loci were monomorphic in Helgoland, compared to the other populations, in which a maximum of one monomorphic locus was observed (Table A7).

3.2.3 | Genetic structure

Genetic differentiation was significant for each pairwise population comparison (p = .003 for all pairs; FSTAT) with an average F_{ST} value of 0.3795 (Table A8), while the strongest differentiation occurred between Helgoland and Tromsø and the weakest between Helgoland and Roscoff. Structure analyses results show that the optimal number of genetic clusters was K = 2 according to the method of Evanno et al. (2005) (Figure A5). We detected a clear hierarchical distinction in genetic structure between two groups (Figure 7a) of northern populations (Spitsbergen, Tromsø, Bodø) and southern populations (Helgoland, Roscoff, Quiberon). A subsequent analysis run separately for northern and southern populations revealed distinct structuring between the three populations present in each subset (Figure 7b,c; K = 3). While gene flow between populations is generally very weak, the relatively highest connectivity occurred between the adjacent Roscoff and Quiberon populations. Additionally, a difference between northern and southern populations is visible at Lo454-27 (Table A7), where one allele is fixed for all southern populations.

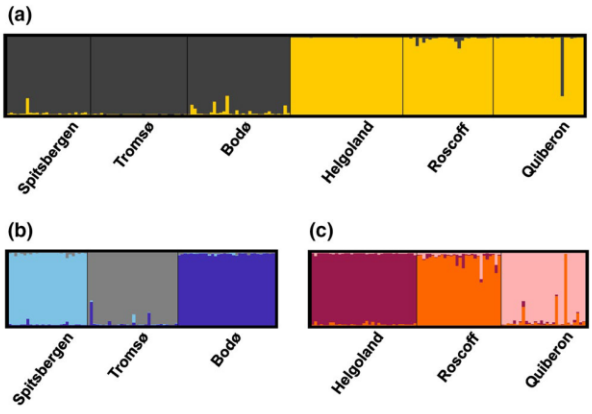

FIGURE 7 Structure bar plot of *Laminaria digitata* populations along the entire distribution range. (a) First hierarchical level of structure obtained for K = 2 genetic clusters. (b) Second hierarchical level of structure for northern populations and (c) second hierarchical level of structure for southern populations of *L. digitata* obtained for K = 3 genetic clusters. Individuals (vertical bars) were assigned probabilities of belonging to clusters (colors) based on differences in genetic variance

3.2.4 | Reproductive system

L. digitata from Tromsø and Helgoland did not show any significant departure from random mating (F_{IS}). We identified $F_{IS} > 0.1$ for Spitsbergen, Bodø, Roscoff, and Quiberon, (Table 4 for multilocus estimates of F_{IS}; Table A7 for single locus estimates of F_{IS}). However, when p-values were corrected for multiple testing ($p < .0069$, FSTAT), heterozygote deficiency was significant only for Bodø and Roscoff.

4 | DISCUSSION

We identified a uniform growth limit across European *Laminaria digitata* populations following a short-term application of 23°C, which conforms with previous studies (Bolton & Lüning, 1982; tom Dieck, 1992). Despite this, we observed slight deviations in magnitude and onset of stress responses among *L. digitata* populations at the cold and warm range margins. Arctic Spitsbergen material presented the strongest heat stress reaction. On the other hand, the two populations naturally experiencing summer temperatures near their upper long-term survival limit, Helgoland and Quiberon, showed moderate advantages in stress responses and growth during the heat treatments. We therefore provide further evidence for the existence of thermal ecotypes of *L. digitata* (King et al., 2019) across the species' entire Northeast Atlantic distribution. The strong genetic structuring of *L. digitata* within northern and southern clades might have facilitated phenotypic divergence, while neutral genetic diversity was not connected to clear patterns of genetic drift or maladaptation along *L. digitata*'s latitudinal distribution.

4.1 | Similarities in growth and biochemical responses along the latitudinal gradient

Growth responses among our tested populations suggest that the upper temperature tolerance limit of *Laminaria digitata* is uniform along its European latitudinal distribution. Growth is an integrative parameter of all metabolic processes and can thus be interpreted as a proxy for organismal stress response. We observed that growth almost completely ceased in the 23°C treatment for all populations (Figure 3), while all populations showed signs of recovery from 21°C when transferred to 15°C (Figure A1). The populations of Tromsø and Spitzbergen showed significantly lower overall growth rates than the southern populations. The lower growth rates of the Arctic populations might be related to prevailing local environmental conditions during sampling (e.g., long day lengths, cold temperature) which may influence growth rates and circannual rhythmicity in kelps (Olischläger & Wiencke, 2013; Schaffelke & Lüning, 1994). Still, results of our study using

meristematic disks of wild adult *L. digitata* material support previous studies using laboratory-cultivated whole juvenile *L. digitata* sporophytes, which also showed uniform upper temperature limits on both sides of the Atlantic and Spitsbergen (Bolton & Lüning, 1982; Franke, 2019; tom Dieck, 1992).

The definition of thermal limits across populations strongly depends on the experimental design (e.g., cultivation conditions and sample age, among other independent variables) and on the response variables measured. Previous studies using photosynthesis (Helgoland: Lüning, 1984) and tissue damage (Nova Scotia: Simonson et al., 2015) as proxies defined the upper thermal tolerance of wild *L. digitata* sporophytes at 18°C to 20°C in experiments that lasted one week. Higher temperatures of 21°C (Simonson et al., 2015) and 23°C (Lüning, 1984) were lethal. However, common-garden experiments demonstrated the capacity for cultivated and wild juvenile *L. digitata* sporophytes from these locations to survive temperatures >20°C for at least one week using growth and occurrence of tissue bleaching as proxies (Helgoland and Nova Scotia: Bolton & Lüning, 1982; tom Dieck, 1992; Nova Scotia: Wilson et al., 2015). Physiological responses may also differ depending on the treatment duration. Whereas maximum quantum yield (F_v/F_m) of Southern English *L. digitata* decreased over a period of 16 days at 18°C (Hargrave et al., 2017), F_v/F_m was stable at 19°C over a shorter period of seven days in our experiment. Still, the reduced growth at 18°C in Hargrave et al. (2017) matches the decrease in growth at 19°C in our study. Thus, uniformity or differences in thermal limits among populations can only be reliably assessed under common-garden conditions, for example, as performed here.

In addition to the strong similarities in the upper thermal limits of growth in our study, carbon contents (Figure 5b) and chlorophyll *a* contents (Figure 6a) did not differ between temperature treatments at all. In contrast, the overall trend of increasing mannitol contents at high temperatures (Figure 5a) has been described for *Saccharina latissima* (Davison & Davison, 1987) and might be linked to the seasonal increase in kelp mannitol storage in summer during the period of slow growth (Haug & Jensen, 1954; Schiener et al., 2015), which, in wild sporophytes, is followed by a peak of the long-term storage compound laminarin in autumn (Haug & Jensen, 1954; Schiener et al., 2015).

The consistent responses of growth and biochemical contents across populations reported here indicate a strong acclimation potential of *L. digitata*'s metabolism to high temperature. Acclimation to wide temperature ranges would reduce selective pressure of temperature in the wild and might explain the small magnitude of local differentiation observed in this study.

4.2 | Differences in growth and photosynthetic parameters among marginal populations

Despite the stability of the upper thermal growth limit, we observed subtle physiological differences in the common-garden heat stress experiment, mainly in the marginal populations of Spitsbergen, Helgoland, and Quiberon. Maximum quantum yield of photosystem II was most sensitive to thermal stress at 21°C and 23°C in Spitsbergen material (Figure 4; Figure A2). This is concordant with the subarctic to Arctic regional climate and provides first evidence for a loss of function in a leading-edge *L. digitata* population, but whether this represents an adaptive trait is yet unknown. Generally, very few cold-temperate algae occurring in the Arctic show true adaptations to the Arctic climate compared to their Atlantic populations (Bischoff & Wiencke, 1993; Wiencke et al., 1994), possibly because the Arctic did not provide a sufficiently stable environment for adaptive evolutionary processes to occur (Wiencke et al., 1994).

At the southern range edge, a slight advantage of Quiberon material to grow at elevated temperatures became evident in the growth response at 19°C during the heat treatment, and in the full recovery from the 21°C treatment (Figure 3; Figure A1). In contrast, photoacclimative responses suggest that the marginal population on the island of Helgoland was most resistant to heat stress. Photosystem II of Helgoland material was minimally impaired by 23°C (Figure 4). Additionally, reactions of xanthophyll pigments (Figure 6b,c) were significantly weaker in Helgoland material than other populations. Increased xanthophyll contents may indicate a photoprotective acclimation reaction (Latowski, Kuczyńska, & Strzałka, 2011; Pfündel & Bilger, 1994; Uhrmacher et al., 1995), while the de-epoxidation ratio of xanthophyll cycle pigments represents the current capacity to quench excessive energy from the photosystem (Pfündel & Bilger, 1994). Helgoland material did not show a significant increase in xanthophyll pigments and presented significantly lower de-epoxidation ratios and therefore lower nonphotochemical quenching (NPQ_{max}, Figure A4) than all other populations. Therefore, the two populations growing in the warmest of the tested locations, which may experience >4 week long periods of mean in situ temperatures of 18°C to 19°C in summer (Helgoland: Bartsch, Vogt, Pehlke, & Hanelt, 2013; Wiltshire et al., 2008; Quiberon: Oppliger et al., 2014; Valero, unpubl.), showed slight physiological advantages to short-term heat exposure in growth and stress responses.

The southernmost populations of Quiberon and Roscoff were curiously the only populations with significantly reduced tissue nitrogen contents in the heat treatments (Figure 5c). A variety of factors including temperature affects nutrient uptake and consequently tissue nitrogen contents, which could be species-specific (Roleda & Hurd, 2019). Therefore, published studies on the impacts of heat stress on nitrogen uptake and storage in kelps differ in their reports of decreased (Gerard, 1997), unaffected (Nepper-Davidsen et al., 2019), or increased nitrogen contents (Wilson et al., 2015). Whether the underlying cause of reduced nitrogen during heat in our study is adaptive, maladaptive, or neutral toward heat resilience in the southern populations remains unclear until further investigation.

4.3 | Population genetics in relation to physiological thermal responses

Population genetics suggest that the slight phenotypic divergence of *L. digitata* might have been facilitated through phylogeographic

separation into two clades and low genetic connectivity between populations. The hierarchical division into a northern and a southern clade in the Northeast Atlantic (Figure 7a) is likely due to postglacial recolonization by two distinct genetic groups located in refugia proposed for the Armorican/Celtic Sea (Brittany and South West UK) and a potential northern refugium at the west coast of Ireland and/or Scotland (Neiva et al., 2020; see also King et al., 2020). Currently, the highest genetic diversity ($H_e \geq 0.6$) published for *L. digitata* populations was observed in Scotland (King et al., 2019, 2020), Northwest Ireland (Neiva et al., 2020), and Northeast Ireland (Brennan et al., 2014), which all exceeded the genetic diversity of the populations investigated in this study. Due to a lack of data, it remains unclear whether a potential glacial refugium of *L. digitata* also corresponds to the well-described Southwest Ireland refugium proposed for many marine species (Kettle, Morales-Muñiz, Roselló-Izquierdo, Heinrich, & Vøllestad, 2011; Provan & Bennett, 2008).

Populations at the "leading edge" (high latitude) are said to be associated with low genetic diversity due to recolonization processes following the Last Glacial Maximum (Hampe & Petit, 2005; for marine seaweeds of the North Atlantic see Assis, Serrão, Claro, Perrin, & Pearson, 2014; Neiva et al., 2016; Provan & Maggs, 2012). Therefore, effects of genetic drift (e.g., depleted genetic diversity, increased inbreeding) may be expected to reduce physiological function in these populations. Here, genetic diversity characteristics of *L. digitata* at its northern range limit (i.e., Spitsbergen) were not significantly lower compared to the other populations in this study and were similar to other Northern Norwegian populations (Neiva et al., 2020). A similar pattern was observed for another Arctic to cold-temperate kelp species, *Saccharina latissima* (Guzinski, Mauger, Cock, & Valero, 2016). Therefore, rather than effects of genetic drift, a lack of selection pressure in the Arctic might have led to a potential reduction of heat tolerance at the northern distribution limit (i.e., relaxed selection; Lahti et al., 2009; Zhen & Ungerer, 2008).

Probably due to the continuous rocky substrata along the Brittany coast, connectivity may be maintained between Quiberon and neighboring populations, which may explain a certain level of gene flow between Roscoff and Quiberon via stepping stone habitats (Figure 7c). Low gene flow can reduce inbreeding depression and associated deleterious effects and may facilitate local adaptation at this southern range edge (Fitzpatrick & Reid, 2019; Sanford & Kelly, 2011). Genetic diversity characteristics for Brittany *L. digitata* populations in this study comply with previous reports (Oppliger et al., 2014; Robuchon et al., 2014). Compared to Roscoff, genetic diversity of *L. digitata* from the island of Helgoland was significantly lower. The population's reduced genetic diversity can be partly explained by genetic isolation due to habitat discontinuity as Helgoland is a rocky substrate surrounded by continuous sandy seafloor (Reichert, Buchholz, & Giménez, 2008). This may rather suggest maladaptation due to less effective selection (such as in *Fucus serratus*; Pearson et al., 2009). However, samples from Helgoland presented the weakest heat stress response in this study. Therefore, we can hypothesize either that historically greater diversity/connectivity was reduced via isolation and drift after resilience to local conditions was established, or that strong selective forces toward the upper thermal limit of *L. digitata* have counterbalanced the effect of genetic drift.

Significant departures from random mating were only observed for the populations of Bodø and Roscoff (F_{IS}; Table 4) and match the magnitude of recent descriptions for *L. digitata* populations (King et al., 2020; Neiva et al., 2020). The higher F_{IS} values in Roscoff *L. digitata* in our study compared to the nearby population of Santec (Robuchon et al., 2014) might be explained by the distance of >1 km between sites, which may already cause substantial variation in F_{IS} (Billot, Engel, Rousvoal, Kloareg, & Valero, 2003). In contrast, the higher F_{IS} values of Quiberon *L. digitata* in our study compared to Oppliger et al. (2014) who sampled at the same location (Pointe de Conguel North) may be an artifact of differing microsatellite markers or might indicate a change in the reproductive system over time (Oppliger et al., 2014; Valero et al., 2011). In all cases, in the absence of data on reproductive ecology, the underlying causes remain speculative.

4.4 | Outlook

The mechanistic temperature treatments applied in this study do not represent realistic temperature scenarios for all tested populations, especially not for the northern clade. However, during our sampling period in August 2018, acute heat spikes surpassed 20°C on twelve days on Helgoland, and on nine days in Quiberon in the shallow sublittoral (in situ data; Bartsch, unpubl.; Valero, unpubl.). Also in South England, *L. digitata* already encounters marine heatwaves reaching 20°C (Burdett, Wright, & Smale, 2019; Joint & Smale, 2017). According to predictions of ocean warming (Müller et al., 2009) and marine heatwaves (Oliver et al., 2018), *L. digitata* will possibly encounter prolonged summer periods of 21°C–23°C at its warm distribution limit until the end of the century.

As a low intertidal to shallow sublittoral species, *L. digitata* is not only threatened by increasing summer SST and marine heatwaves (Bartsch et al., 2013; Hargrave et al., 2017), but also by other stressors during emersion such as desiccation and warm air temperature (Hereward, King, & Smale, 2020; King, Wilcockson, et al., 2018), high irradiance, and UV radiation (Gruber, Roleda, Bartsch, Hanelt, & Wiencke, 2011; Roleda, Hanelt, & Wiencke, 2006). These multiple stressors are most likely responsible for the die-off event of Helgoland *L. digitata* sporophytes after exposure to SST > 19°C over a prolonged period of 11 days (Bartsch et al., 2013). Additionally, rising temperatures may negatively affect sporophyte and gametophyte reproduction (sporogenesis: Bartsch et al., 2013; gametogenesis: Lüning, 1980; Martins, Tanttu, Pearson, Serrão, & Bartsch, 2017) which might contribute to range contractions of *L. digitata*. Therefore, despite the slight physiological advantages we identified in the southern populations, *L. digitata* is threatened by a substantial loss of genetic diversity at its current southern distribution limit (King et al., 2020; Neiva et al., 2020; Oppliger et al., 2014; Robuchon et al., 2014).

Models have predicted a northward shift of the entire distribution range of *L. digitata* until 2100 in the RCP 8.5 emission scenario, implying possible extinction of southern populations, including Roscoff and Quiberon (Assis et al., 2018; Raybaud et al., 2013). A potential loss of more heat-tolerant populations at the trailing edge and a simultaneous expansion of northern, slightly less heat-tolerant *L. digitata* phenotypes implies that global warming might drive a decrease in the overall adaptive capacity to warming in the kelp *Laminaria digitata*. Neutral genetic diversity was recently described as an indicator for heat resilience of kelp populations by indicating physiological versatility among individuals (Wernberg et al., 2018). Conversely, marine heatwaves can deplete the genetic diversity of kelp populations in strong selective bottleneck events (Gurgel, Camacho, Minne, Wernberg, & Coleman, 2020). Therefore, response variability among individuals shapes the adaptive capacity of populations to withstand bottleneck events and to allow directional selection (Chevin, Lande, & Mace, 2010; Kelly, 2019; King, McKeown, et al., 2018). This implies that genetically depleted populations (e.g., marginal populations) are at even higher risk of extinction. A recent study showed high phenotypic variation among five genotypes of Helgoland *L. digitata* (Liesner et al., 2020), but studies correlating inter-individual response variation to genetic diversity across populations are necessary to investigate the implications of genetic diversity for population resilience during climate change.

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CONFLICT OF INTEREST

All authors declare that they are free of competing interests.

AUTHOR CONTRIBUTION

Daniel Liesner: Conceptualization (supporting); Data curation (equal); Formal analysis (lead); Investigation (lead); Methodology (supporting); Project administration (supporting); Visualization (lead); Writing-original draft (lead). **Louise Fouqueau:** Data curation (equal); Formal analysis (supporting); Investigation (supporting); Visualization (supporting); Writing-original draft (supporting); Writing-review & editing (equal). **Myriam Valero:** Data curation (equal); Formal analysis (supporting); Funding acquisition (equal); Resources (supporting); Writing-review & editing (equal). **Michael Y. Roleda:** Conceptualization (supporting); Formal analysis (supporting); Writing-review & editing (equal). **Gareth A. Pearson:** Conceptualization (supporting); Formal analysis (supporting); Writing-review & editing (equal). **Kai Bischof:** Resources (supporting); Supervision (supporting); Writing-review & editing (equal). **Klaus Valentin:** Funding acquisition (equal); Resources (supporting); Supervision (supporting); Writing-review & editing (equal). **Inka Bartsch:** Conceptualization (lead); Formal analysis (supporting); Funding acquisition (equal); Investigation (supporting); Project administration (lead); Resources (lead); Supervision (lead); Writing-review & editing (equal).

DATA AVAILABILITY STATEMENT

Genotype and physiological data generated during this study are available at platform DRYAD. Genotype data are accessible at https://doi.org/10.5061/dryad.jsxksn06c. Physiological data are accessible at https://doi.org/10.5061/dryad.73n5tb2ts.

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APPENDIX

See Tables A1, A2, A3, A4, A5, A6, A7, A8

TABLE A1 Results of generalized least squares models to examine variability of growth rates and maximum quantum yield of *Laminaria digitata* disks in the detailed time course of the heat stress experiment (Figures A1, A2)

		RGR				F_v/F_m			
Population	Parameter	numDF	denDF	*F*-value	*p*-value	numDF	denDF	*F*-value	*p*-value
Spitsbergen	Temperature	3	60	17.43	**<.0001**	3	72	46.35	**<.0001**
	Time	4	60	72.81	**<.0001**	5	72	9.31	**<.0001**
	Temperature × time	12	60	5.24	**<.0001**	15	72	14.78	**<.0001**
Tromsø	Temperature	3	80	87.90	**<.0001**	3	96	8.87	**<.0001**
	Time	4	80	778517.93	**<.0001**	5	96	45.61	**<.0001**
	Temperature × time	12	80	29.20	**<.0001**	15	96	7.02	**<.0001**
Helgoland	Temperature	3	80	159.35	**<.0001**	3	96	209.26	**<.0001**
	Time	4	80	23.36	**<.0001**	5	96	9198.09	**<.0001**
	Temperature × time	12	80	27.92	**<.0001**	15	96	7.47	**<.0001**
Roscoff	Temperature	3	80	168.27	**<.0001**	3	96	15.32	**<.0001**
	Time	4	80	22.71	**<.0001**	5	96	71.51	**<.0001**
	Temperature × time	12	80	14.12	**<.0001**	15	96	12.45	**<.0001**
Quiberon	Temperature	3	80	271.81	**<.0001**	3	96	14.08	**<.0001**
	Time	4	80	813970892.83	**<.0001**	5	96	67.21	**<.0001**
	Temperature × time	12	80	75.62	**<.0001**	15	96	4.34	**<.0001**

Notes: Fresh weight relative growth rates and maximum quantum yield F_v/F_m over all time points (T-5 (only F_v/F_m), T0, T3, T6, T8, T15) were tested against interactive effects of heat treatment and time for each population separately. Generalized least squares models were performed as described in the methods section, but without the fixed effect for population. Tested values are means of 2 per replicate ($n = 5$, $n = 4$ for Spitsbergen). numDF, numerator degrees of freedom; denDF, denominator degrees of freedom. Statistically significant values are indicated in bold text.

TABLE A2 Results of generalized least squares models to examine variability of photoacclimation parameters of *Laminaria digitata* disks obtained via rapid light curves in the heat stress experiment (Figure A3)

			$rETR_{max}$		I_k		α	
Parameter	numDF	denDF	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Initial values	1	35	0.49	.4877	0.03	.8571	0.54	.4665
Population	4	35	2.32	.0760	2.25	.0831	0.39	.8149
Temperature	3	35	2.87	.0503	0.59	.6284	1.83	.1601
Population × temperature	12	35	1.59	.1413	1.38	.2197	1.04	.4390

Notes: Maximum relative electron transport rate $rETR_{max}$, saturation irradiance I_k, and photosynthetic efficiency α were tested against initial values as covariate and interactive effects of population and heat stress temperature treatment. $n = 3$, $n = 2$ for Spitsbergen. numDF, numerator degrees of freedom; denDF, denominator degrees of freedom. Statistically significant values are indicated in bold text.

TABLE A3 Results of generalized least squares models to examine variability of nonphotochemical quenching parameters of *Laminaria digitata* disks obtained via rapid light curves in the heat stress experiment (Figure A4)

			NPQ_{max}		E_{50}		*n*	
Parameter	**numDF**	**denDF**	***F*-value**	***p*-value**	***F*-value**	***p*-value**	***F*-value**	***p*-value**
Initial values	1	35	78.00	**<.0001**	8.60	**.0059**	0.47	.4980
Population	4	35	20.73	**<.0001**	4.73	**.0037**	2.07	.1063
Temperature	3	35	1.68	.1883	2.14	.1132	11.43	**<.0001**
Population × temperature	12	35	1.86	.0761	1.17	.3397	0.91	.5448

Notes: Maximum nonphotochemical quenching NPQ_{max}, saturation irradiance $E_{50,}$ and sigmoidicity coefficient *n* were tested against initial values as covariate and interactive effects of population and heat stress temperature treatment. $n = 3$, $n = 2$ for Spitsbergen. numDF, numerator degrees of freedom; denDF, denominator degrees of freedom. Statistically significant values are indicated in bold text.

TABLE A4 Correlation coefficients (Kendall's rank correlation tau) and *p*-values in parentheses between relative growth rates (RGR), maximum quantum yield (F_v/F_m), biochemical, and pigment characteristics of *Laminaria digitata* during / after the heat treatment.

	F_v/F_m	Mannitol	Carbon	Nitrogen	C:N ratio	Chl *a*	VAZ : Chl *a*	De-epox.
RGR	0.1176 (0.0903)	**−0.5570 (<0.0001)**	**−0.4218 (<0.0001)**	**−0.2547 (0.0011)**	0.0337 (0.6668)	**0.2013 (0.0082)**	**−0.2911 (0.0001)**	−0.1269 (0.0979)
F_v/F_m		−0.1117 (0.1436)	0.0943 (0.2293)	**0.3068 (<0.0001)**	**−0.3125 (<0.0001)**	−0.1148 (0.1325)	**−0.2828 (0.0002)**	**−0.3954 (<0.0001)**
Mannitol			**0.3691 (<0.0001)**	**0.1895 (0.0154)**	0.0288 (0.7131)	**−0.1785 (0.0191)**	**0.2444 (0.0014)**	0.1035 (0.1769)
Carbon				**0.3053 (<0.0001)**	0.0758 (0.3327)	−0.0912 (0.2436)	0.1186 (0.1323)	**−0.1964 (0.0127)**
Nitrogen					**−0.6189 (<0.0001)**	−0.1418 (0.0700)	−0.0494 (0.5309)	**−0.2317 (0.0033)**
C : N ratio						0.0779 (0.3194)	**0.1928 (0.0144)**	**0.1993 (0.0114)**
Chl *a*							**−0.3431 (<0.0001)**	−0.0841 (0.2729)
VAZ : Chl *a*								**0.3372 (<.0001)**

Notes: $n = 80$, except $n = 96$ for the correlation of RGR and F_v/F_m due to the inclusion of Spitsbergen material. Outliers (see Section 2.2.6) were not included in the analysis and further reduced *n* in the following comparisons: $n = 76$ for comparisons involving data from C:N analysis; $n = 79$ for comparisons involving xanthophyll pigment data; $n = 75$ for comparisons involving both. Statistically significant values are indicated in bold text.

TABLE A5 Frequency of null alleles per marker and per population of *Laminaria digitata* obtained using FREENA software

	Ld148	Ld158	Ld167	Ld371	Ld531	Ld704	Lo454-23	Lo454-24	Lo454-17	Lo454-27	Lo454-28	Lo4-24
Spitsbergen	0.00001	**0.29297**	**0.07023**	**0.17081**	**0.12798**	0.00001	0	**0.07593**	0.001	0.00001	0	0.00836
Tromsø	0	0.001	0	**0.23131**	0.00001	0	0.02599	0	0.00004	**0.10953**	0.00006	0.00001
Bodø	**0.11562**	0.00001	0.00001	**0.13494**	0.00001	0	**0.20983**	**0.11409**	0.00006	0.00001	0.00006	0.00001
Helgoland	0.00012	0	0	0	**0.12754**	0.01587	**0.09471**	0.00007	0.001	0.001	0.00007	0.001
Roscoff	0.01255	**0.17234**	0.04511	0.01372	0.01204	0.02449	0.01144	**0.072**	0	0.001	0.03801	**0.11747**
Quiberon	0.00002	**0.13067**	0	0.00001	0.00001	**0.06379**	0.00001	0.01969	0.01643	0.001	0.00001	**0.13145**

Notes: Significant values (>0.05) are highlighted in bold text.

TABLE A6 *p*-values for linkage disequilibrium based on 7,920 permutations using FSTAT for each pair of markers and for each tested population of *Laminaria digitata*.

	Spitsbergen	Tromsø	Bodø	Helgoland	Roscoff	Quiberon	All
Ld148 × Ld158	0.0601	NA	0.03169	0.72551	0.86768	0.24407	0.12702
Ld148 × Ld167	0.48548	0.22184	0.42841	0.39836	0.85303	0.08611	0.23763
Ld148 × Ld371	0.86351	0.67891	0.67071	0.97235	1.000	0.58561	0.95379
Ld148 × Ld531	0.25341	0.92727	0.03485	0.53889	0.86793	0.64343	0.53876
Ld148 × Ld704	0.63838	0.74798	0.42247	0.73977	0.99242	0.95783	0.99255
Ld148 × Lo454-23	0.57816	0.22096	0.30152	0.04836	0.46035	0.68763	0.15758
Ld148 × Lo454-24	0.64104	0.73687	0.28207	0.23068	0.92172	0.19432	0.56023
Ld148 × Lo454-17	NA	0.71098	0.11995	NA	0.67348	0.49886	0.61439
Ld148 × Lo454-27	0.89369	0.69785	0.36667	NA	NA	NA	0.63232
Ld148 × Lo454-28	0.22159	0.43359	1.000	1.000	0.68952	0.50316	0.50682
Ld148 × Lo4-24	0.03573	0.42323	0.42854	NA	0.54268	0.6423	0.32374
Ld158 × Ld167	0.86679	NA	0.76705	0.02525	0.64356	0.12525	0.29356
Ld158 × Ld371	0.15985	NA	0.69912	0.80833	0.52942	0.08106	0.25694
Ld158 × Ld531	0.00328	NA	0.18043	0.14495	0.9149	0.80038	0.05467
Ld158 × Ld704	0.4952	NA	0.42841	0.32866	0.43321	0.54899	0.45783
Ld158 × Lo454-23	0.02033	NA	0.65568	0.28081	0.50934	0.01995	0.01667
Ld158 × Lo454-24	0.87917	NA	0.64545	1.000	0.59116	0.01414	0.43258
Ld158 × Lo454-17	NA	NA	0.06465	NA	0.22841	0.0178	0.00051
Ld158 × Lo454-27	0.19419	NA	0.71275	NA	NA	NA	0.22614
Ld158 × Lo454-28	0.19078	NA	1.000	0.20543	0.63182	0.65328	0.3702
Ld158 × Lo4-24	0.70341	NA	0.87551	NA	0.67866	0.20783	0.62247
Ld167 × Ld371	0.36174	0.00025	1.000	0.34293	1.000	0.3322	0.00455
Ld167 × Ld531	0.05758	0.56717	0.55909	0.04444	0.40821	0.97942	0.13775
Ld167 × Ld704	0.83005	0.60568	0.95101	0.2303	0.99886	0.00379	0.80126
Ld167 × Lo454-23	0.18662	0.69457	0.38965	0.05707	0.13788	0.26174	0.05972
Ld167 × Lo454-24	0.30391	0.91503	0.85732	1.000	0.71831	0.03157	0.51402
Ld167 × Lo454-17	NA	0.69116	0.33434	NA	0.79356	0.16944	0.3524
Ld167 × Lo454-27	0.92083	0.32462	0.25682	NA	NA	NA	0.58106
Ld167 × Lo454-28	0.34066	1.000	0.35177	0.49015	0.36124	0.86629	0.43093
Ld167 × Lo4-24	0.47664	0.32159	0.68194	NA	0.85593	0.09508	0.4077
Ld371 × Ld531	0.76705	0.26389	0.40669	0.72311	0.58157	0.36275	0.47146
Ld371 × Ld704	0.84331	0.36035	0.50631	0.46111	0.11136	0.25328	0.27121
Ld371 × Lo454-23	0.89356	0.8221	0.68662	0.1923	1.000	0.95732	0.84381
Ld371 × Lo454-24	0.83624	0.32134	0.31301	0.40543	0.94672	0.04836	0.29495
Ld371 × Lo454-17	NA	0.31957	0.77841	NA	0.84066	0.1779	0.3404
Ld371 × Lo454-27	0.35467	0.23144	0.92778	NA	NA	NA	0.45985
Ld371 × Lo454-28	0.4375	0.57008	0.60114	1.000	0.89609	0.0928	0.37576
Ld371 × Lo4-24	0.42058	0.2971	0.53636	NA	0.775	0.1327	0.22071
Ld531 × Ld704	0.81288	0.81149	0.12412	0.38674	0.15543	0.61932	0.36818
Ld531 × Lo454-23	0.07538	0.13788	0.54545	0.84091	0.11351	0.06641	0.03737
Ld531 × Lo454-24	0.19268	0.92639	0.82361	1,000	0.59975	0.55025	0.81225
Ld531 × Lo454-17	NA	0.84457	0.15303	NA	0.97071	0.49293	0.71755
Ld531 × Lo454-27	0.47929	0.31073	0.73043	NA	NA	NA	0.31742
Ld531 × Lo454-28	0.34861	1.000	1.000	1.000	0.30404	0.90366	0.75455

(Continues)

TABLE A6 (Continued)

	Spitsbergen	Tromsø	Bodø	Helgoland	Roscoff	Quiberon	All
Ld531 × Lo4-24	0.52273	0.57109	0.19495	NA	0.12828	0.91831	0.5899
Ld704 × Lo454-23	0.50972	0.00947	0.53687	0.19646	0.44167	0.7471	0.13674
Ld704 × Lo454-24	0.4298	0.14419	0.69066	1.000	0.72083	0.80783	0.59609
Ld704 × Lo454-17	NA	0.68649	0.74924	NA	1.000	0.67487	0.92891
Ld704 × Lo454-27	0.11439	0.72702	0.63068	NA	NA	NA	0.41111
Ld704 × Lo454-28	0.24886	1.000	0.52702	1,000	0.44937	0.15909	0.32626
Ld704 × Lo4-24	0.70265	0.45909	0.84861	NA	1.000	0.67361	0.88182
Lo454-23 × Lo454-24	0.92702	0.65505	0.90543	0.65492	0.51919	0.31389	0.86705
Lo454-23 × Lo454-17	NA	0.4721	0.34811	NA	0.83005	0.6101	0.70707
Lo454-23 × Lo454-27	0.47083	0.65593	0.36629	NA	NA	NA	0.54091
Lo454-23 × Lo454-28	0.69482	0.72866	0.34167	0.06098	0.74419	0.9197	0.76465
Lo454-23 × Lo4-24	0.35202	0.8721	0.85316	NA	0.55833	0.36427	0.81338
Lo454-24 × Lo454-17	NA	0.21427	1.000	NA	0.96174	0.09684	0.32399
Lo454-24 × Lo454-27	0.23636	0.75164	1.000	NA	NA	NA	0.82412
Lo454-24 × Lo454-28	1,000	1,000	0.21465	1.000	0.89381	0.88232	0.96944
Lo454-24 × Lo4-24	0.11982	0.37437	0.07917	NA	0.40707	0.09482	0.02134
Lo454-17 × Lo454-27	NA	0.72412	1.000	NA	NA	NA	0.77513
Lo454-17 × Lo454-28	NA	1.000	1.000	NA	0.23914	0.63902	0.61717
Lo454-17 × Lo4-24	NA	0.6721	1.000	NA	0.1197	0.00745	0.05101
Lo454-27 × Lo454-28	0.02109	0.06742	1.000	NA	NA	NA	0.00303
Lo454-27 × Lo4-24	0.86869	0.84432	1.000	NA	NA	NA	0.98295
Lo454-28 × Lo4-24	0.51023	1.000	0.50189	NA	1.000	0.41717	0.70063

Notes: Microsatellite loci published for *Laminaria digitata* (Ld; Billot et al., 1998) and *Laminaria ochroleuca* (Lo; Coelho et al., 2014). The *p*-value after multiple testing correction for 5% nominal level is 0.000126. No linkage disequilibrium is significant in the dataset.

TABLE A7 Estimates of genetic diversity and deviation from random mating for each locus and each population of *Laminaria digitata* tested in this study.

Population	Locus	*n*	N_a	AR	P_a	H_e	H_o	F_{IS}
Spitsbergen	Ld148	26	3	2.808	0	0.212	0.231	−0.110
	Ld158	25	4	4	1	0.709	0.200	0.712
	Ld167	26	5	4.802	1	0.714	0.615	0.121
	Ld371	26	9	8.355	0	0.796	0.500	0.360
	Ld531	26	3	2.966	1	0.520	0.308	0.396
	Ld704	21	2	2	0	0.136	0.143	−0.077
	Lo454-23	26	5	4.61	0	0.531	0.615	−0.182
	Lo454-24	26	5	4.581	0	0.281	0.231	0.164
	Lo454-17	26	1	1	0	0.000	0.000	#NV
	Lo454-27	26	2	2	0	0.510	0.538	−0.077
	Lo454-28	26	3	2.808	0	0.446	0.500	−0.144
	Lo4-24	26	2	2	0	0.483	0.462	0.025
Tromsø	Ld148	30	3	3	0	0.603	0.800	−0.350
	Ld158	30	1	1	0	0.000	0.000	#NV
	Ld167	30	4	3.883	1	0.274	0.300	−0.113
	Ld371	30	9	8.456	1	0.793	0.367	0.530
	Ld531	30	3	2.89	0	0.159	0.167	−0.068
	Ld704	30	4	3.89	0	0.393	0.467	−0.209
	Lo454-23	30	5	4.614	0	0.667	0.600	0.086
	Lo454-24	30	4	3.976	0	0.444	0.500	−0.146
	Lo454-17	30	2	2	0	0.398	0.400	−0.023
	Lo454-27	30	2	1.976	0	0.097	0.033	0.649
	Lo454-28	30	2	1.7	1	0.033	0.033	−0.017
	Lo4-24	30	4	3.975	0	0.569	0.533	0.047
Bodø	Ld148	32	4	3.963	1	0.634	0.438	0.299
	Ld158	32	3	2.882	0	0.203	0.188	0.061
	Ld167	31	8	7.664	2	0.839	0.839	−0.016
	Ld371	31	15	12.946	1	0.871	0.613	0.285
	Ld531	32	3	2.874	0	0.177	0.156	0.101
	Ld704	32	5	4.884	1	0.684	0.688	−0.021
	Lo454-23	32	6	5.538	0	0.635	0.313	0.500
	Lo454-24	32	5	4.542	1	0.590	0.438	0.246
	Lo454-17	32	2	1.656	0	0.031	0.031	−0.016
	Lo454-27	32	2	1.999	0	0.173	0.188	−0.103
	Lo454-28	32	2	1.656	0	0.031	0.031	−0.016
	Lo4-24	32	3	2.963	0	0.549	0.594	−0.098
Helgoland	Ld148	34	2	2	0	0.504	0.500	−0.008
	Ld158	35	3	2.6	1	0.520	0.600	−0.169
	Ld167	35	4	3.687	0	0.512	0.600	−0.189
	Ld371	35	9	7.861	0	0.618	0.657	−0.078
	Ld531	35	4	3.937	0	0.493	0.314	0.353
	Ld704	35	2	2	0	0.487	0.457	0.048
	Lo454-23	35	3	2.843	0	0.535	0.371	0.295
	Lo454-24	35	2	1.6	0	0.029	0.029	−0.014
	Lo454-17	35	1	1	0	0.000	0.000	#NV
	Lo454-27	35	1	1	0	0.000	0.000	#NV

(Continues)

TABLE A7 (Continued)

Population	Locus	*n*	N_a	AR	P_a	H_e	H_o	F_{IS}
	Lo454-28	35	2	1.6	0	0.029	0.029	−0.014
	Lo4-24	35	1	1	0	0.000	0.000	#NV
Roscoff	Ld148	28	7	6.678	2	0.782	0.750	0.023
	Ld158	28	4	3.749	1	0.477	0.250	0.466
	Ld167	28	7	6.718	2	0.727	0.643	0.100
	Ld371	28	15	14.025	0	0.906	0.893	−0.003
	Ld531	28	4	3.997	0	0.658	0.571	0.115
	Ld704	28	4	3.691	0	0.475	0.429	0.081
	Lo454-23	28	9	8.211	1	0.779	0.714	0.067
	Lo454-24	28	4	3.737	1	0.410	0.286	0.290
	Lo454-17	28	2	2	0	0.299	0.357	−0.217
	Lo454-27	28	1	1	0	0.000	0.000	#NV
	Lo454-28	28	2	2	0	0.249	0.214	0.125
	Lo4-24	28	3	2.691	0	0.105	0.036	0.652
Quiberon	Ld148	28	6	5.497	0	0.664	0.643	0.014
	Ld158	28	3	2.941	0	0.257	0.143	0.434
	Ld167	28	5	4.441	0	0.318	0.321	−0.031
	Ld371	28	11	9.927	1	0.836	0.786	0.043
	Ld531	28	4	3.737	0	0.497	0.500	−0.023
	Ld704	28	3	2.75	0	0.408	0.321	0.199
	Lo454-23	28	7	6.191	1	0.519	0.536	−0.050
	Lo454-24	28	6	5	1	0.344	0.321	0.047
	Lo454-17	28	4	3.999	1	0.603	0.607	−0.025
	Lo454-27	28	1	1	0	0.000	0.000	#NV
	Lo454-28	28	2	2	0	0.503	0.536	−0.084
	Lo4-24	28	3	2.75	0	0.328	0.179	0.446

Notes: Locus, microsatellite loci published for *Laminaria digitata* (Ld; Billot et al., 1998) and *Laminaria ochroleuca* (L_o; Coelho et al., 2014); *n*, number of individuals for which the marker amplified; N_a, number of observed alleles; AR, allelic richness standardized for equal sample size (21 individuals); P_a, number of private alleles per locus; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{IS}, fixation index (inbreeding coefficient) of individuals with respect to local subpopulation; #NV, no calculation of F_{IS} in monomorphic loci. Note that in Helgoland, Roscoff and Quiberon, the locus Lo454-27 is fixed while it is polymorphic for Spitsbergen, Tromsø and Bodø. This explains why this locus was not included in the study of Robuchon et al. (2014).

TABLE A8 Fixation index (F_{ST}) for each pair of the *Laminaria digitata* populations tested in this study

	Tromsø	Bodø	Helgoland	Roscoff	Quiberon
Spitsbergen	0.422	0.344	0.468	0.338	0.444
Tromsø		0.293	0.582	0.466	0.520
Bodø			0.442	0.319	0.385
Helgoland				0.156	0.352
Roscoff					0.162

Notes: All *p*-values obtained with 300 permutations using FSTAT were 0.003 and therefore significant (the *p*-value corrected for multiple testing is .003).

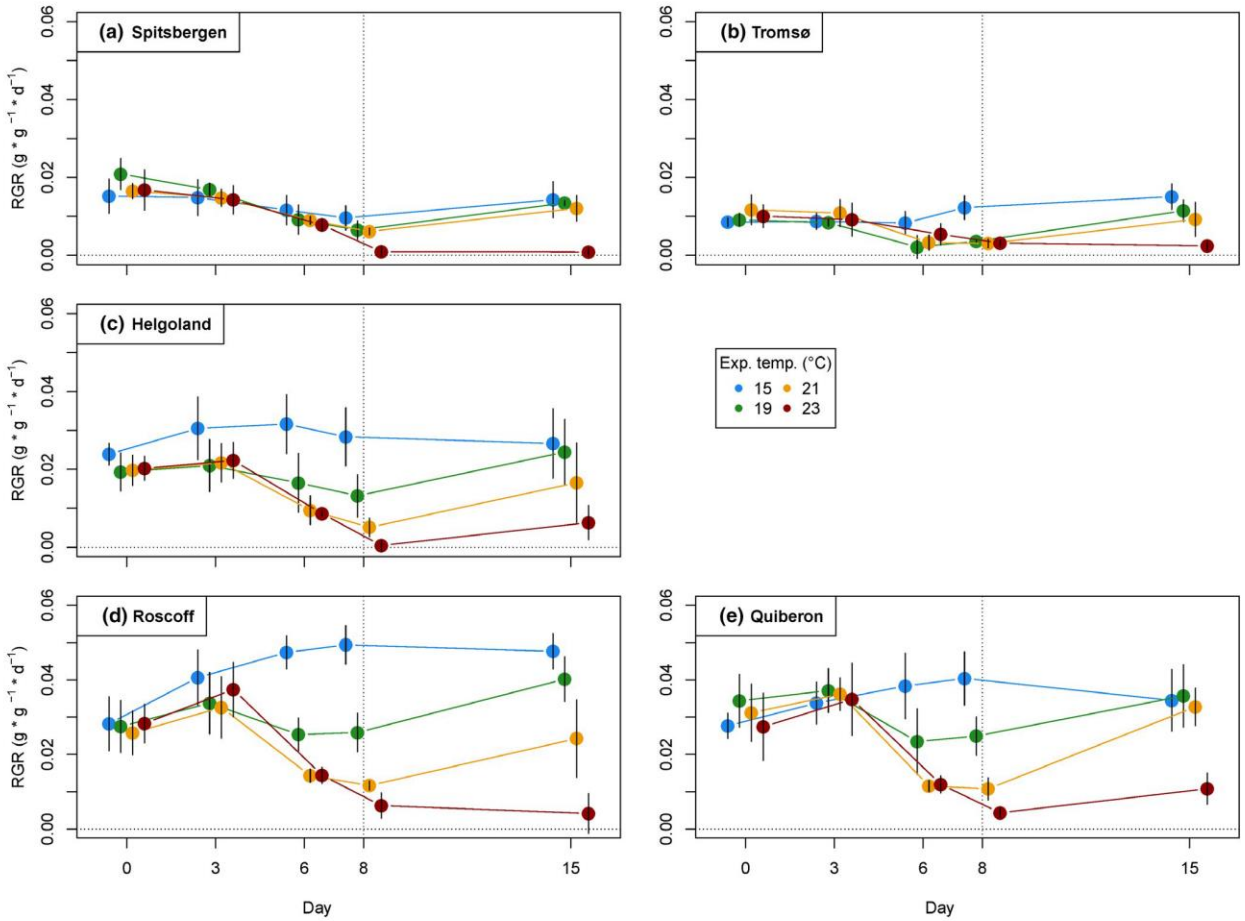

FIGURE A1 Relative growth rates (RGR) of *Laminaria digitata* disks from (a) Spitsbergen, (b) Tromsø, (c) Helgoland, (d) Roscoff, and (e) Quiberon over the heat stress experiment. Points represent growth rates between subsequent measuring days. Mean values ± SD ($n = 5$, for Spitsbergen $n = 4$). Points at day 0 represent growth over acclimation at 15°C, the end of the heat treatment at day 8 is marked with a vertical dotted line, and zero growth is marked with a horizontal dotted line. For statistical analysis, see Table A1.

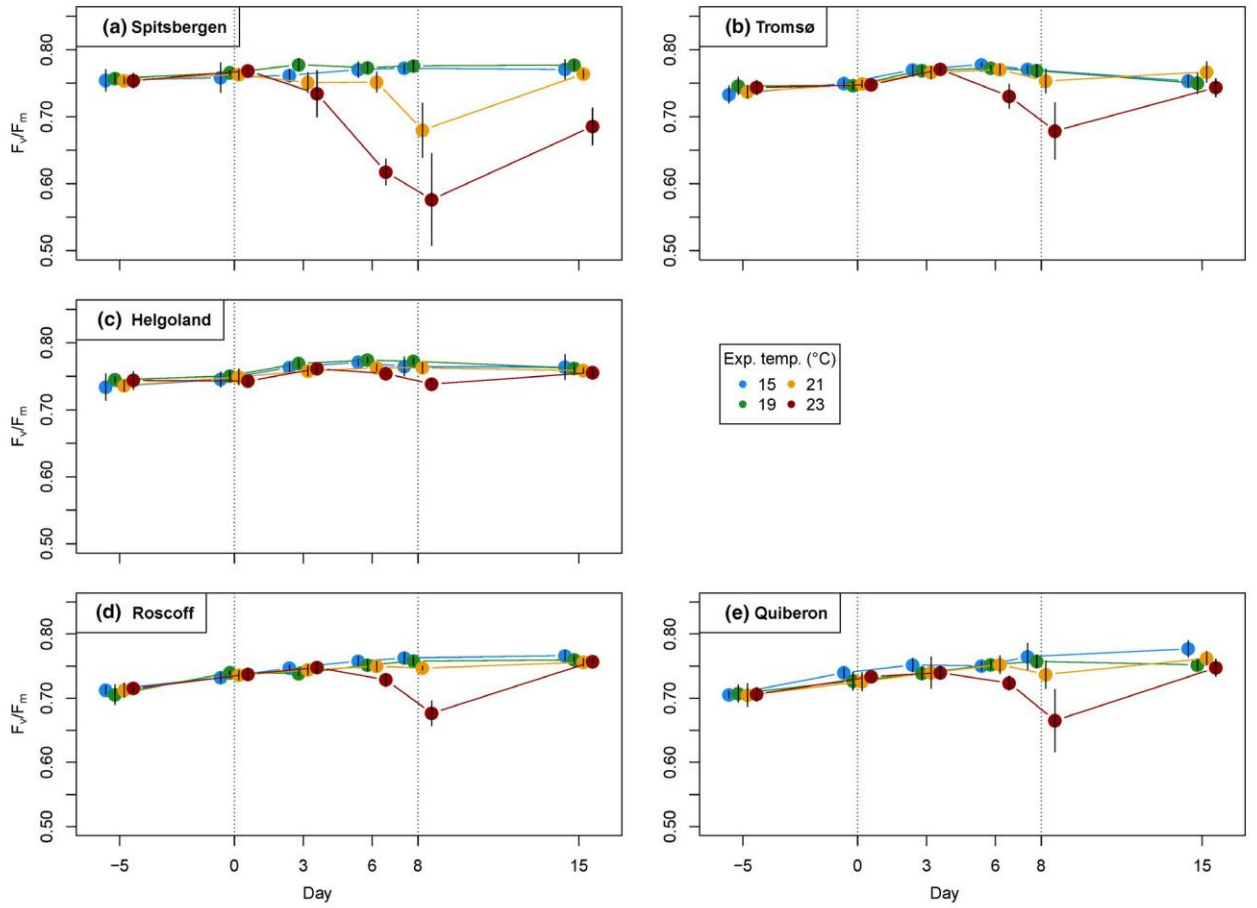

FIGURE A2 Maximum quantum yield (F_v/F_m) of *Laminaria digitata* disks from (a) Spitsbergen, (b) Tromsø, (c) Helgoland, (d) Roscoff, and (e) Quiberon over the heat stress experiment. Mean values ± SD ($n = 5$, for Spitsbergen $n = 4$). End of the acclimation at 15°C and end of the heat treatment are marked with dotted lines. For statistical analysis, see Table A1.

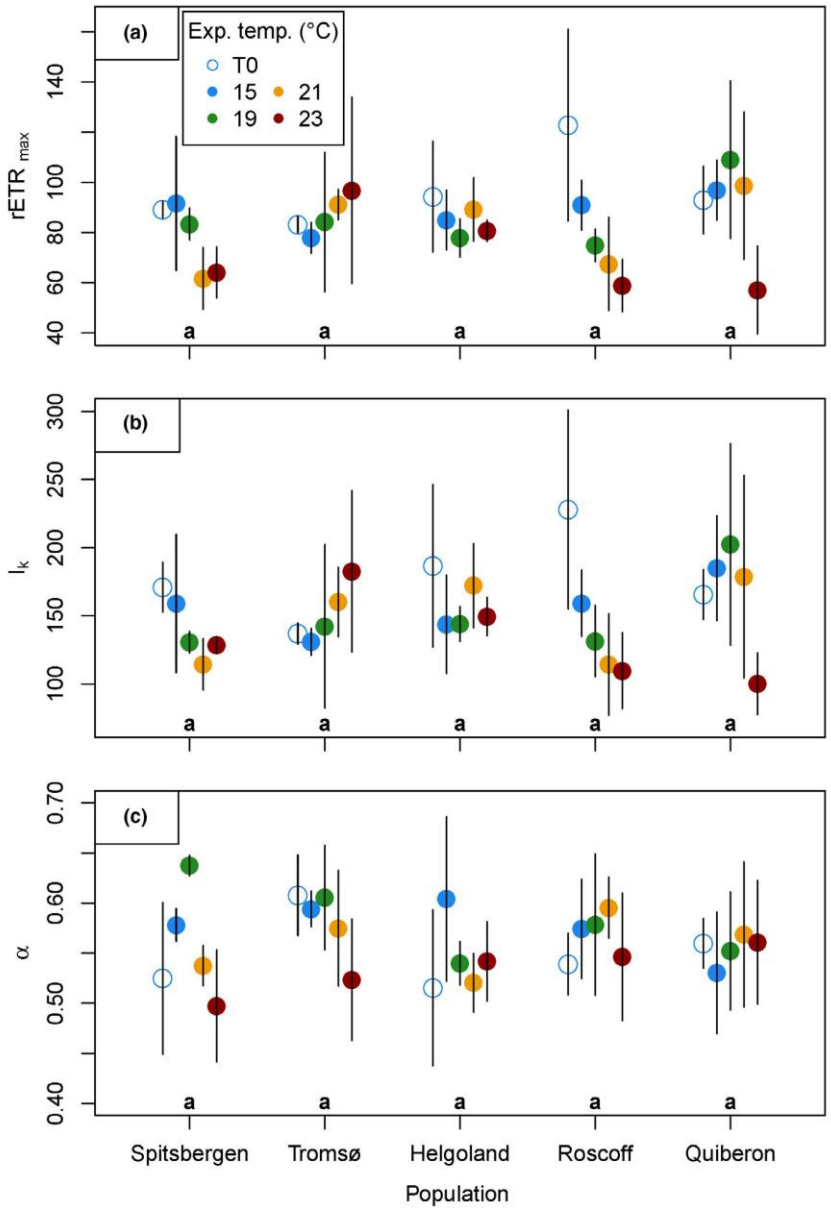

FIGURE A3 Photoacclimation parameters of *Laminaria digitata* disks obtained via rapid light curves after acclimation at 15°C (day 0, empty circles) and after the heat treatment (day 8, colored points). (a) Maximum relative electron transport rate $rETR_{max}$ (relative unit), (b) saturation irradiance I_k (µmol photons m^{-2} s^{-1}), (c) photosynthetic efficiency α (rETR/µmol photons m^{-2} s^{-1}). Mean values ± SD ($n = 3$, for Spitsbergen $n = 2$). Analyses of variance returned no significant differences between populations (indicated by lowercase letters) and temperatures (Table A2).

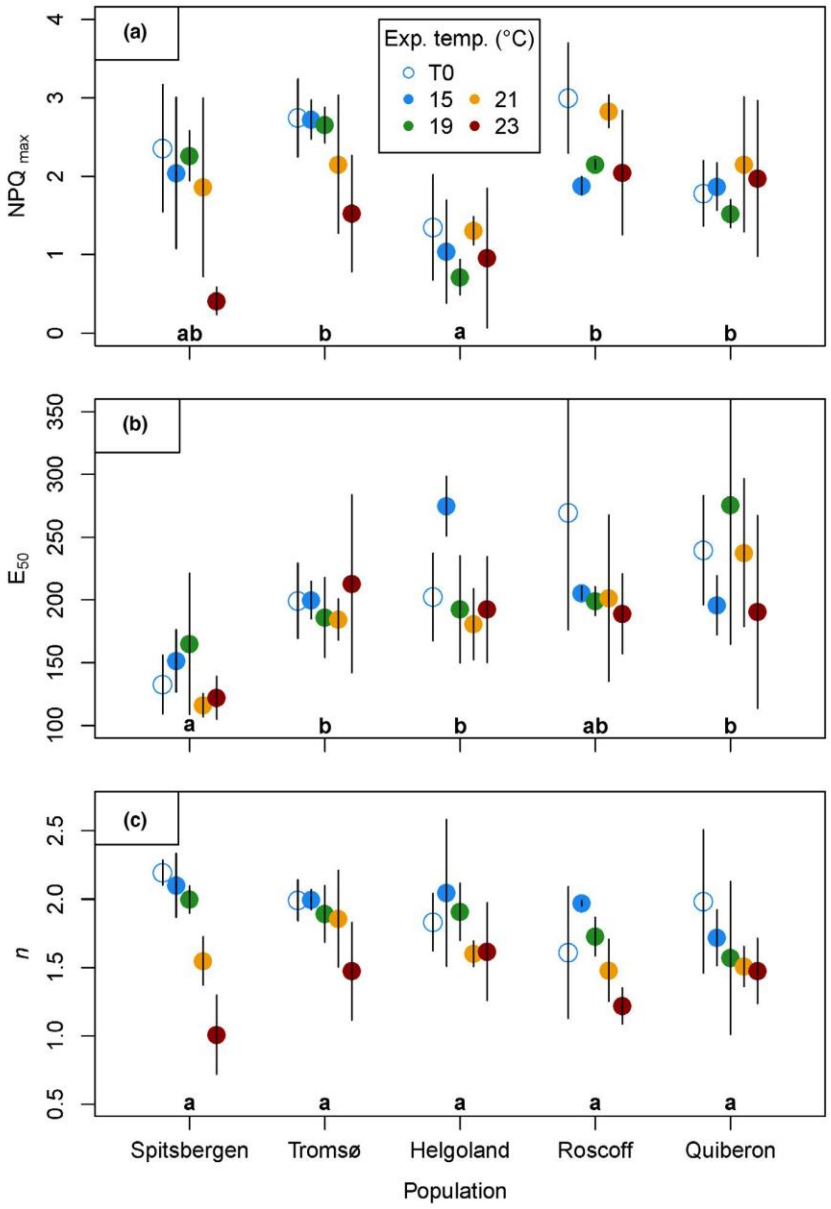

FIGURE A4 Nonphotochemical quenching parameters of *Laminaria digitata* disks obtained via rapid light curves after acclimation at 15°C (day 0, empty circles) and after the heat treatment (day 8, colored points). (a) Maximum nonphotochemical quenching NPQ_{max} (relative unit), (b) saturation irradiance E_{50} (µmol photons m^{-2} s^{-1}), (c) sigmoidicity coefficient *n* (unitless). Mean values ± *SD* ($n = 3$, for Spitsbergen $n = 2$). Significant differences between mean population responses are indicated by lowercase letters (Table A3; Tukey tests, $p < .05$).

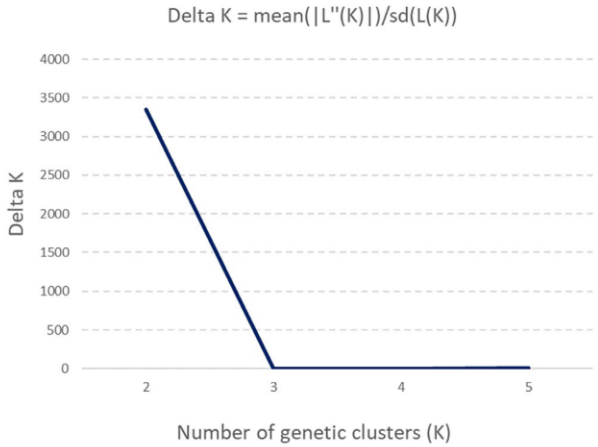

FIGURE A5 ΔK (Evanno et al., 2005) plotted against K, associated with K = 2 to K = 5 obtained with Structure Harvester during the analysis of genetic structure of *Laminaria digitata* populations

4 Publication II

Daniel Liesner, Lisa N. S. Shama, Nora Diehl, Klaus Valentin, Inka Bartsch

Thermal plasticity of the kelp *Laminaria digitata* (Phaeophyceae) across life cycle stages reveals the importance of cold seasons for marine forests

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Thermal Plasticity of the Kelp *Laminaria digitata* (Phaeophyceae) Across Life Cycle Stages Reveals the Importance of Cold Seasons for Marine Forests

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Phenotypic plasticity (genotype × environment interaction) is an especially important means for sessile organisms to cope with environmental variation. While kelps, the globally most productive group of seaweeds, generally possess a wide thermal performance range, kelp populations at their warm distribution limits are threatened by ocean warming. Here, we investigated effects of temperature during ontogeny of the kelp *Laminaria digitata* across haploid gametophyte and diploid sporophyte life cycle stages in five distinct genetic lines. We hypothesized that thermal plasticity increases trait performance of juvenile sporophytes in experimental temperatures that match the temperature experienced during gametogenesis and recruitment, and that plasticity differs among genetic lines (genetic variation for plasticity). We applied a full-factorial experimental design to generate different temperature histories by applying 5 and 15°C during meiospore germination, gametogenesis of parental gametophytes and recruitment of offspring sporophytes (19–26 days), and juvenile sporophyte rearing (91–122 days). We then tested for thermal plasticity among temperature history treatments at 5 and 15°C in a final 12-day experiment assessing growth, the storage compound mannitol, carbon and nitrogen contents, and fluorometric responses in 3–4 month old sporophytes for five genetic lines. Our study provides evidence for the importance of cold temperatures at early development on later sporophyte performance of *L. digitata*. Gametogenesis and recruitment at 5°C promoted higher growth of offspring sporophytes across experimental temperatures. While photosynthetic capacity was higher at 15°C, carbon and nitrogen storage were higher at 5°C, both showing fast acclimation responses. We identified an important role of genetic variation for plasticity in shaping *L. digitata*'s thermal plasticity. Trait performance at 5 or 15°C (reaction norm slopes) differed among genetic lines, even showing opposite response patterns. Interestingly, genetic variation for plasticity was only significant when sporophytes were reared at 5°C. Thus, we provide evidence that the cold-temperate to Arctic kelp species, *L. digitata*, which possesses a wide temperature tolerance between 0 and 23°C, is impaired by warm temperature during gametogenesis and recruitment, reducing growth of juvenile sporophytes and expression of variable thermal plasticity in the wild.

Keywords: phenotypic plasticity, temperature acclimation, developmental plasticity, carry-over effect, biphasic life cycle, gametogenesis, ontogeny, genetic variation

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INTRODUCTION

In a changing environment, organisms have few mechanisms to cope with temporal habitat heterogeneity. In order to prevent range contractions, populations can move according to their environmental requirements (i.e., range shifts) or they can acclimate and/or adapt to the new conditions, all of which are interactive processes within a complex response framework (Donelson et al., 2019). Especially for sessile species, phenotypic plasticity is an important means of quick response to environmental change. Phenotypic plasticity has been studied for decades (Bradshaw, 1965; Sultan, 1995; Chevin et al., 2010; Fox et al., 2019), and describes phenotypic changes in an individual in response to its environment (genotype × environment interaction). This includes fast and reversible acclimation responses, but also carry-over effects due to exposure during early ontogeny which persist during development (COE, also developmental plasticity; Palmer et al., 2012; Byrne et al., 2020). Additionally, the environment experienced by parents can influence offspring traits regardless of offspring environment (parental effects *sensu* Salinas et al., 2013). Importantly, environmental change cues may elicit different plastic responses among genotypes (genetic variation for plasticity; Newman, 1994; Nicotra et al., 2010), thereby increasing trait variability within a population that selection can act on. These concepts highlight the importance of taking into account environmental history and investigating multiple genotypes when assessing thermal plasticity of populations and species.

Brown seaweeds of the order Laminariales (i.e., kelps *sensu stricto*) are especially important habitat builders along warm-temperate to polar rocky shorelines (Lüning, 1990). They provide a three-dimensional, species-rich and highly productive habitat, the marine forest (Wernberg and Filbee-Dexter, 2019), which is globally under threat by rising sea temperatures especially at their equatorward margins (Wernberg et al., 2016; Vergés et al., 2019). Many kelps have a broad thermal performance range spanning 20°C or more (e.g., Lüning, 1984; tom Dieck, 1993; Wiencke et al., 1994), within which their metabolism can quickly adjust (i.e., acclimate) and prevent irreversible stress responses. This high acclimation capacity allows kelps to persist along environmental gradients by fast adjustment of e.g., photosynthesis (Davison and Davison, 1987; Davison et al., 1991; Rothäusler et al., 2011), carbon metabolism (Scheschonk et al., 2019), or pigment contents (Li et al., 2019; Mabin et al., 2019b) in response to a variety of factors. All laminarian kelps alternate between independent generations of haploid gametophytes and diploid sporophytes in their life cycle. Therefore, kelps provide a useful experimental system to investigate thermal plasticity across ontogeny. We consider the kelp germline to encompass the whole process between meiosis and production of eggs and sperm (see also Grossniklaus, 2011; Schmidt et al., 2015). Therefore, the majority of the kelp germline is contained in the autonomous gametophyte generation, which allows for experimental control of the germline environment from meiospore release to zygote formation. Further, this facilitates breeding of gametophytes obtained from one sporophyte, allowing comparisons among genetic lines and tests of genetic variation for plasticity.

In this study, we investigated thermal plasticity of the kelp *Laminaria digitata* (Hudson) J.V. Lamouroux, which is a key habitat-former in the upper sublittoral and infralittoral fringe of cold-temperate and Arctic rocky coasts (Lüning, 1990; Dankworth et al., 2020). In the North Atlantic, *L. digitata* is one of four kelp species of the genus *Laminaria* (among other kelp genera), which occur across marine biogeographical regions (*sensu* Lüning, 1990): *L. solidungula* is an Arctic species; *L. digitata* occurs in Arctic and cold-temperate regions; *L. hyperborea* is a cold-temperate species and co-occurs along most of its distribution with *L. digitata* in the sublittoral; and *L. ochroleuca* is a warm-temperate species and co-occurs with *L. digitata* only in Brittany and South England (Smale et al., 2015). Along the European coast, *L. digitata* occurs between the 0°C February isotherm on the archipelago of Spitsbergen and the 18°C August isotherm in southern Brittany, France (for isotherms, see Müller et al., 2009). In Brittany, the species marginally extends into the warm-temperate region (February isotherm > 10°C *sensu* Lüning, 1990). Despite a high gametophyte temperature tolerance of 23°C over several weeks (tom Dieck, 1993), models predict the loss of the southernmost *L. digitata* populations due to ocean warming (Raybaud et al., 2013; Assis et al., 2018).

At our study location on the North Sea island of Helgoland (Germany), *L. digitata* meiospore release occurs mainly between May and November (Bartsch et al., 2013), but it is not known when gametogenesis and recruitment of juvenile sporophytes are most prevalent, especially as vegetative gametophytes might provide a perennial "seed bank" (tom Dieck, 1993; Edwards, 2000). Therefore, temperatures might vary greatly during formation of primordial germ cells in gametophytes and during subsequent recruitment and growth of juvenile offspring sporophytes. Despite *L. digitata*'s potentially broad thermal performance spectrum, a relatively warm temperature of 15°C was shown to enhance gametogenesis, sporophyte growth and sporogenesis (tom Dieck, 1992; Bartsch et al., 2013; Martins et al., 2017; Franke, 2019) compared to a cool temperature of 5°C, whereas sporophyte recruitment was most successful under cool conditions (Martins et al., 2017). Importantly, it is unknown whether and how temperature variation during early life stages of gametogenesis and recruitment influences thermal plasticity of juvenile sporophytes.

Generally, temperature responses of *L. digitata* are not static, but may vary across seasons (Lüning, 1984) and are shaped by endogenous and annual abiotic changes. For example, the endogenous growth rhythmicity present in laminarian kelps is modulated and synchronized by changes in daylength (Lüning, 1991; tom Dieck, 1991; Schaffelke and Lüning, 1994), but the influence of rhythmicity and daylengths on the temperature performance of kelps is unknown. Daylength and temperature may control the seasonal accumulation of the carbon storage compounds mannitol and laminarin in Laminariales (Schaffelke, 1995), which naturally peak in late summer (Black, 1954; Schiener et al., 2015; Manns et al., 2017). Additionally, few indications for carry-over and parental effects have been shown in kelp and fucoid seaweeds. Li and Brawley (2004) demonstrated a positive parental effect of warm receptacle environment on

Fucus vesiculosus embryo survival during heat stress. Mabin et al. (2019a) demonstrated significant variation in morphology between lineages of *Ecklonia radiata* gametophytes at different temperatures and irradiances, which they attributed to potential parental and genetic effects. The latter further indicates that individual phenotypic variation may play a substantial role in shaping response variability of kelp populations. Phenotypic differences among genotypes have been shown to modulate plasticity in terrestrial plants (Galloway, 2001; Suter and Widmer, 2013), but similar research in kelps does not exist to our knowledge.

The main objective of this study was to investigate thermal plasticity of juvenile *Laminaria digitata* sporophytes in the context of their temperature history across ontogeny and among genotypes. Our approach tested for effects of temperature treatments of 5 and 15°C over the kelp life cycle on thermal plasticity of juvenile *L. digitata* sporophytes. First, we assessed growth at 5 and 15°C across multiple seasons using wild adult sporophytes to relate our experimental results to adult sporophyte responses in the wild. We then released meiospores from five wild sporophytes, cultivated them separately as genetic lines and produced four temperature history treatments by applying 5 and 15°C during meiospore germination, gametogenesis and sporophyte recruitment, and during growth of juvenile sporophytes. In a final 12-day experiment, we split 3–4 month old centimeter-sized sporophytes again between 5 and 15°C, and assessed thermal plasticity of growth, the storage compound mannitol, carbon and nitrogen contents, and fluorometric parameters. We hypothesized that trait performance of juvenile sporophyte offspring would increase in experimental temperatures that matched the temperature experienced during gametogenesis and recruitment (COE or developmental plasticity) in comparison to mismatching temperature history treatments (match-mismatch approach; Engqvist and Reinhold, 2016). We further hypothesized that genetic lines would differ in their capacity for thermal plasticity (genetic variation for plasticity), potentially contributing to thermal response variability of *L. digitata* populations under ocean warming.

MATERIALS AND METHODS

Experimental Design

To assess seasonal thermal plasticity of adult sporophytes in the wild, we first followed growth of field-collected sporophyte meristems at 5 and 15°C at three time points over the year (experiment 1, **Figure 1**). At these temperatures, sporophyte growth is in its optimum range (Bolton and Lüning, 1982) and gametogenesis is not inhibited (tom Dieck, 1992; Martins et al., 2017).

Following this, we tested for effects of temperature during early ontogeny on trait plasticity of juvenile offspring *L. digitata*

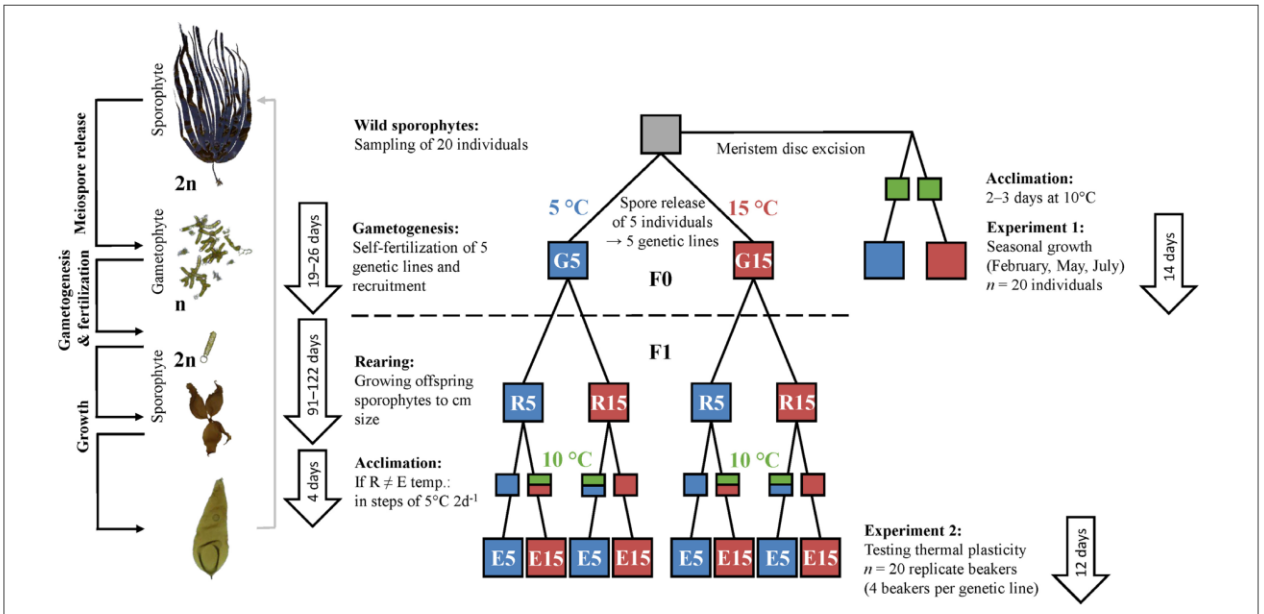

FIGURE 1 | Experimental design to test for thermal plasticity of wild adult sporophytes (experiment 1) and juvenile sporophytes (experiment 2) of *Laminaria digitata*. Twenty fertile *L. digitata* sporophytes were sampled in the field (Helgoland, North Sea). Excised meristems from 20 individuals sampled in May, July 2017 and February 2018 were used for experiment 1. Meiospores of five sporophytes were released and genetic lines were maintained by self-fertilization in 5 and 15°C (gametogenesis, 19–26 days). Throughout the flowchart for experiment 2, each colored box represents five separate genetic lines. Following recruitment, each gametogenesis treatment was divided to rear juvenile microscopic sporophytes at 5 and 15°C (rearing, 91–122 days). An acclimation period of 4 days was applied for all treatments, with stepwise temperature change of 2°C d^{-1} for treatments in which rearing and experimental temperatures differed. Experiment 2 was conducted with 7–11 cm long sporophytes from five genetic lines at 5 and 15°C for 12 days. The schematic life cycle represents life stages at the different treatment steps of the experiment.

sporophytes. Here, we employed a full-factorial experimental design using five genetic lines of *L. digitata* at two experimental temperatures (5 and 15°C), and encompassing parent (F0) and offspring (F1) generations at three time periods: during gametogenesis and recruitment (F0/F1), early sporophyte (F1) rearing, and growth of F1 juvenile sporophytes (experiment 2; **Figure 1**). This full-factorial experimental approach on distinct genetic lines allowed disentanglement of the roles of genetic background and environmental effects, as well as assessments of genetic variation for plasticity (Herman and Sultan, 2016; Donelson et al., 2018).

Sampling and Cultivation of *Laminaria digitata*

We collected 20 fertile *L. digitata* adult sporophytes from the low intertidal zone on the island of Helgoland (North Sea, Germany; 54.1779 N, 7.8926 E) on 10 May 2017, 10 July 2017 and 04 February 2018. For the seasonal growth experiment (experiment 1), two discs (Ø 24 mm) were cut from each sporophyte meristem at a distance of 5 cm from the stipe-blade transition zone and transported to the laboratory wet and cool. In July, we also sampled fertile blade parts to be used for meiospore release in the main experiment on thermal plasticity of juvenile sporophytes (experiment 2). The use of blade tissue samples is an established experimental method to gain approximations of whole-organism responses (Graiff et al., 2015; Hargrave et al., 2017; King et al., 2019). For example, excised meristematic tissue can be used to investigate growth activity and photosynthesis (Buchholz and Lüning, 1999; Scheschonk et al., 2019), and vegetative blade tissue can be used to induce sporogenesis (Bartsch et al., 2013).

All samples were cultivated in 10 mL L^{-1} Provasoli-enriched sterilized natural seawater (PES; Provasoli, 1968; modifications: HEPES-buffer instead of TRIS, double concentration of Na_2glycerophosphate, iodine enrichment after Tatewaki, 1966) in temperature-controlled chambers (5, 10, 15°C, variation ± 1°C). The light:dark photoperiod of 16 h:8 h applied in this study induces constant, year-round growth in *L. digitata* (Schaffelke and Lüning, 1994). Irradiance varied depending on the life cycle stage to accommodate the irradiance requirements of each stage (Lüning, 1980; tom Dieck, 1992; Han and Kain, 1996): 30–40 μmol photons m^{-2} s^{-1} for meristem discs and juvenile sporophytes, and 16–18 μmol photons m^{-2} s^{-1} for gametophytes (ProfiLux 3 with LED Mitras daylight 150, GHL Advanced Technology, Kaiserslautern, Germany). Sporophytes and meristem discs were cultivated in aerated one liter glass beakers filled with 800 mL PES.

Experiment 1: Seasonal Growth of Wild *Laminaria digitata* Sporophytes

Growth of meristem discs at 5 and 15°C was assessed in February, May and July (**Figure 1**, experiment 1). Discs were first acclimated to laboratory conditions for two to three days at 10°C (n = 20 individuals each held separately). Subsequently, two discs per individual were divided between 5 and 15°C in a paired design (**Figure 1**). Medium in the beakers was changed weekly. After 14 days, we assessed area growth via image analysis (WinFolia Pro 2006a software; Regent Instruments Inc., Quebec, Canada). Absolute area growth rates were calculated with a linear formula:

$$AGR\ (cm^2 * d^{-1}) = \frac{x_2 - x_1}{t_2 - t_1}$$

where x_1 = area at time 1, x_2 = area at time 2, t_1 = time 1 in days (d), t_2 = time 2 in days. We also measured quantum yield of photosystem II (F_v/F_m) as a stress parameter in the center of each disc using pulse-amplitude modulation fluorometry (PAM-2100, Walz, Effeltrich, Germany) after 5 min dark-acclimation.

Meiospore Release, Gametogenesis, Recruitment, and Rearing

Sori sampled in July were stored for 1–2 days in plastic bags at <5 μmol photons m^{-2} s^{-1} in sterilized natural seawater (SW) prior to meiospore release. Meiospores were released from sori following the method of Bartsch (2018). Meiospores from five adult individuals were sowed separately into plastic dishes. These five genetic lines were followed separately throughout the experiment. Each set of meiospores was immediately split to germinate, grow, complete gametogenesis and recruit microscopic sporophytes at 5 and 15°C (gametogenesis treatment: G5 and G15; **Figure 1**).

Gametogenesis in *L. digitata* is a gradual process stretching over one to several weeks depending on environmental conditions (Lüning, 1980). In our experiment, following settlement overnight, meiospores germinated within four days and further developed into one- to few-celled male and female free-living gametophytic filaments, which subsequently became fertile. Fertile female gametophytes extrude single eggs which remain loosely attached to the discharged oogonium (Schreiber, 1930). Free-swimming spermatozoids produced by male gametophytes subsequently fertilize the eggs (Lüning, 1980). The resulting zygote is the initial cell of the next generation, the diploid F1-offspring sporophyte, which is physically separated from the parental gametophyte generation (Schreiber, 1930).

Because not all gametophyte cells undergo gametogenesis simultaneously, we ensured saturation of sporophyte recruitment within each gametogenesis temperature to impede sporophyte recruitment from remaining vegetative gametophytes in the following F1 rearing step. In our experiment, most female gametophytes had released their eggs after 19 days at 15°C and after 26 days at 5°C. At this time, the recruited sporophytes were between one and seven days old. Following saturation, recruited microscopic F1 sporophytes were again divided into 5 and 15°C to enable growth to a sufficient length of several cm for experiment 2 (rearing treatment: R5 and R15; **Figure 1**). The rearing phase took between 91 and 122 days, and produced four temperature history pre-treatments (G5–R5, G5–R15, G15–R5, G15–R15).

Experiment 2: Thermal Plasticity in Juvenile *Laminaria digitata* Sporophytes

Experiment 2 was conducted in two consecutive runs to reduce the workload at one time and to accommodate slower sporophyte

growth at 5°C. The first run included all sporophytes reared at 15°C and the second run, which started 24 days later, included all sporophytes reared at 5°C. Sporophytes reared at 5°C had a mean length of 11 cm and sporophytes reared at 15°C had a mean length of 7 cm at the start of the experiment. We accounted for differences in rearing time and initial length in the statistical analyses (see below). At this point, the five genetic lines from four temperature histories were again divided into 5 and 15°C (E5 and E15, resulting in eight experimental treatment groups; **Figure 1**, experiment 2) to assess their thermal plasticity in contrasting temperature environments (Sultan, 2004). When the experimental temperature differed from the rearing temperature, we first acclimated sporophytes for two days at 10°C followed by two days at the target temperature to reduce acute temperature stress responses in the experiment. When rearing and experimental temperatures matched, sporophytes were transferred to the experimental setup without a change of temperature and held for four days prior to the start of the experiment (**Figure 1**, acclimation). For each genetic line in the eight temperature history groups, four replicate beakers each containing five sporophytes (n = 20 replicates per treatment group, N = 160 experimental units in total) were held in 1.7 L PES in aerated plastic (PETG) containers. The medium was exchanged every three days over the course of acclimation and the 12-day experiment.

Growth

For experiment 2, photos were taken after acclimation (day 0) and at the end of the experiment (day 12), and lengths of sporophytes were measured by tracing a central line along each sporophyte using the segmented line tool in ImageJ 1.51j8 (Schneider et al., 2012). Length sums of the five sporophytes per beaker were used to assess growth (n = 4 replicate beakers per experimental treatment and genetic line).

Biochemistry

We assessed mannitol content (the primary photosynthetic product and compatible solute; Davison and Reed, 1985; Groisillier et al., 2014) to estimate carbon assimilation by sporophytes. Analyses of elemental carbon and nitrogen provided insight into assimilation and nutrient storage, while C:N ratios allowed interpretations of nutrient sufficiency (Hurd et al., 1996; Rosell and Srivastava, 2004). Before acclimation (day -4), 3–22 sporophytes from each genetic line and temperature history were pooled to reach sufficient dry weight (> 200 mg), deep-frozen in liquid nitrogen and stored at −80°C (n = 5 genetic lines per treatment; N = 20 samples in total). After the experiment (day 12), two sporophytes from each experimental replicate (n = 20 replicates per experimental treatment; N = 160 samples in total) were deep-frozen. All samples were lyophilized and ground to a fine powder. Mannitol was extracted in 70% ethanol from three subsamples (10–15 mg) of each experimental replicate and analyzed following the HPLC method described by Karsten et al. (1991). Means of the three subsamples of each mannitol replicate were used in the statistical analysis. For carbon and nitrogen analysis, samples of 2–3 mg for carbon and nitrogen were combusted at 1000°C in an elemental analyzer (EURO EA, HEKAtech GmbH, Wegberg, Germany) with acetanilide as standard.

Fluorometry

Temperature can influence photosynthesis by mediating pigment, enzyme or photosystem contents (Gerard and Du Bois, 1988; Davison et al., 1991; Machalek et al., 1996; Li et al., 2019). Therefore, we tested for variation of fluorometric parameters in response to temperature treatments as a measure of photo-acclimation. One sporophyte per replicate was randomly chosen for fluorometric measurements (using a PAM-2100 device) before temperature acclimation (day -4) and at the end of experiment 2 (day 12). After 5 min dark acclimation, optimum quantum yield of photosystem II (F_v/F_m) was measured in the basal meristematic region of the sporophytes, directly followed by rapid light curves (RLC) with irradiance steps between 0–511 μmol photons m^{-2} s^{-1}. Based on the photon flux density (PFD) and the effective quantum yield, relative electron transport rates (rETR) in photosystem II were calculated as rETR = PFD * Yield (Maxwell and Johnson, 2000; Hanelt, 2018). rETR was plotted against PFD, and the resulting rETR vs. irradiance curves were fit following the model of Jassby and Platt (1976) to calculate maximum relative electron transport rate $rETR_{max}$, saturation irradiance I_k, and photosynthetic efficiency α of each curve.

Statistics

Because of bleaching of three thalli during experiment 2, we removed three replicates from all analyses (leading to total N = 157, and n = 18 and n = 19 in one treatment group each). For the growth analysis, one more replicate was removed after the loss of two sporophytes from a replicate beaker (N = 156). All analyses were performed in the R statistical environment (R version 3.6.0; R Core Team, 2019). Linear mixed effects models were fit using the lme function within the "nlme" package (Pinheiro et al., 2019) including a weights argument to incorporate the variance structure (Zuur et al., 2009). Normal distribution of standardized residuals was assessed visually via Q-Q plots and histograms, and non-normality was treated by log-transformation (Underwood, 1997). Factor significance was assessed via analyses of covariance (ANCOVA) and p-values were corrected for multiple testing following the false discovery rate approach (FDR; Benjamini and Hochberg, 1995).

In experiment 1, meristem disc growth rates and F_v/F_m were modeled using temperature treatment, month, and their interaction as fixed effects, with initial values of area and F_v/F_m as respective covariates, random intercepts for individuals (area and F_v/F_m models), and random slopes for experimental temperature (area model only). *Post hoc* pairwise comparisons of least-squares means (Tukey adjusted) between treatments were performed using the "emmeans" package (Lenth, 2019).

In experiment 2, we fit separate linear mixed effects models for each rearing temperature (i.e., experimental run) to account for any potentially confounding effects of assaying the two rearing temperatures at different times. Response parameters were modeled using initial values of each parameter as covariates, temperature treatment steps and their interaction (gametogenesis * experimental temperature) as fixed effects,

random intercepts for genetic line, and random slopes for experimental temperature. We tested for significance of gametogenesis temperature (temperature effects during early ontogeny), experimental temperature (temperature acclimation within the sporophyte stage), and their interaction (modulation of sporophyte thermal plasticity by gametogenesis temperature) using two-way ANCOVA. Significance of random factors was assessed using likelihood ratio tests between full (random slope for experimental temperature and random intercept for genetic line) and stepwise reduced models (random intercept for genetic line, and no random argument).

RESULTS

Experiment 1: Seasonal Growth of Wild *L. digitata* Sporophytes

Seasonal growth (**Figure 2A**) and optimum quantum yield (F_v/F_m; **Figure 2B**) of wild *L. digitata* meristem tissue were significantly influenced by experimental temperature, sampling month, and their interaction (**Table 1**). Mean initial absolute growth rates between days 0 and 3 (data not shown) decreased over seasons from 0.39 $cm^2\ d^{-1}$ in February to 0.16 $cm^2\ d^{-1}$ in May to 0.09 $cm^2\ d^{-1}$ in July, and thereby reflected known seasonal growth responses of *L. digitata* (Kain, 1979; Lüning, 1979). While in February and July growth rates over 14 days were significantly higher at 15°C than at 5°C (Tukey tests, $p < 0.001$), there were no significant differences between growth at 5°C and 15°C in May (Tukey test, $p = 0.996$; **Figure 2A**; for individual growth rates see **Supplementary Table S1**). Mean daily sea surface temperatures (SST) over 14 days before sampling in February, May and July were 5.8°C ± 1.2°C, 8.6°C ± 0.8°C, and 16.1°C ± 1.3°C, respectively (Helgoland Roads data; Wiltshire et al., 2008). Mean optimum quantum yield (F_v/F_m; **Figure 2B**) ranged between 0.7 and 0.8 across seasons, and was always significantly higher at 15°C compared to 5°C ($p < 0.0001$; **Table 1**), while mean F_v/F_m was lowest at 5°C in July.

Experiment 2: Thermal Plasticity in Juvenile *L. digitata* Sporophytes

Due to the different rearing temperatures, initial values for most parameters differed at the start of experiment 2 (**Supplementary Table S2**), which we accounted for by including them as model covariates and by separating the statistical analyses by rearing temperature. We show residual growth based on linear models of final length as a function of initial length for each rearing temperature in **Figure 3** (absolute growth rates are given in the text and **Supplementary Figure S1**). In **Figure 3**, the dotted zero-line represents predicted values based on initial length and deviations from this line represent temperature effects. All other response parameters are shown as absolute values. For

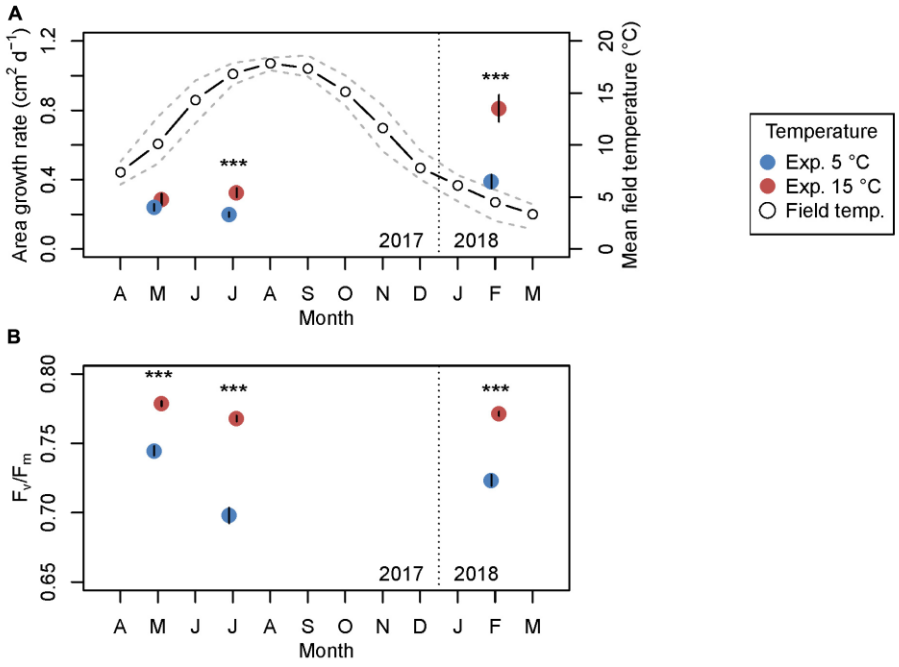

FIGURE 2 | Seasonal growth of wild adult *Laminaria digitata* meristem discs sampled in May and July 2017, and February 2018 cultivated at 5 and 15°C for 14 days (experiment 1). **(A)** Absolute area growth rate over 14 days (primary y-axis, mean values ± SE, $n = 20$) and field sea-surface temperature around Helgoland (secondary y-axis). Open circles represent mean temperatures per month and dashed gray lines delimit minimum and maximum monthly temperatures for the year 2017/2018 (Helgoland Roads data, Wiltshire et al., 2008). **(B)** Quantum yield F_v/F_m at the end of the 14-day experiment (mean values ± SE, $n = 20$). ***$p < 0.001$ (Tukey test).

TABLE 1 | Results of linear mixed effects models to examine thermal plasticity of wild adult *Laminaria digitata* meristem discs in the seasonal growth experiment 1.

			Area AGR		F_v/F_m	
Parameter	**numDF**	**denDF**	***F*-value**	***p*-value**	***F*-value**	***p*-value**
Initial values	1	56	163.13	**<0.0001**	19.43	**<0.0001**
Temperature	1	56	201.68	**<0.0001**	219.50	**<0.0001**
Month	2	57	12.17	**<0.0001**	22.66	**<0.0001**
Temperature × month	2	56	28.21	**<0.0001**	9.31	**0.0003**

Absolute area growth rates (AGR) and optimum quantum yield (F_v/F_m) were tested using initial values as covariates, interactive effects of the fixed factors temperature and month, and individual (identity) as a random effect. numDF and denDF denote numerator and denominator degrees of freedom, respectively. Statistically significant values ($p < 0.05$) are indicated in bold.

TABLE 2 | Results of linear mixed effects models to examine thermal plasticity of length growth of juvenile *Laminaria digitata* sporophytes in experiment 2.

				Length	
Rearing	**Parameter**	**numDF**	**denDF**	***F*-value**	***p*-value**
5°C	Initial length	1	69	285.42	**<0.0001**
	Gametogenesis temp.	1	69	16.14	**0.0003**
	Experimental temp.	1	69	0.38	0.5782
	Gameto × experimental temp.	1	69	3.63	0.0859
15°C	Initial length	1	69	1173.81	**<0.0001**
	Gametogenesis temp.	1	69	27.45	**<0.0001**
	Experimental temp.	1	69	5.67	**0.0273**
	Gameto × experimental temp.	1	69	5.85	**0.0264**

Length at the end of experiment 2 was tested using initial length as a covariate and the interactive effects of gametogenesis temperature and experimental temperature as fixed effects. Genetic line and the interaction between genetic line and experimental temperature were analyzed as random effects. The two rearing temperatures were tested in separate models. numDF and denDF denote numerator and denominator degrees of freedom, respectively. p-values were adjusted for multiple comparisons with FDR corrections. Statistically significant values ($p < 0.05$) are indicated in bold.

all parameters in experiment 2, we show reaction norms of traits across experimental temperatures (E5 or E15) color-coded by gametogenesis temperatures (G5 or G15) in two rearing temperature panels (R5 or R15). In this context, we define reaction norms as the relationship (slope) between trait responses at the two experimental treatments E5 and E15.

Growth

Despite the long rearing period (91–122 days), we found significant, persistent effects of gametogenesis and recruitment temperature on growth of 3–4 month old sporophytes (**Figure 3** and **Table 2**). In both rearing temperatures, mean sporophyte growth was significantly higher following G5 (R5: $p = 0.0003$; R15: $p < 0.0001$; **Table 2**), whereas mean growth of sporophytes following G15 remained below the zero prediction across experimental temperatures. Effects of experimental temperature and interactive gametogenesis × experimental temperature effects were only significant in R15 (**Table 2**). The significant main effect of experimental temperature in R15 ($p = 0.0273$; **Table 2**) led to higher overall growth at E15 compared to E5. This indicates an acclimation response, in which growth rates were adjusted to the experimental temperature within 12 days. In R15, the significant gametogenesis × experimental temperature interaction ($p = 0.0264$; **Table 2**) modified sporophyte growth responses. This is shown as higher growth in the G5–R15–E15 treatment (0.30 ± 0.04 cm sporophyte^{-1} d^{-1}, treatment mean ± SD) compared to the other treatments (G5–R15–E5: 0.27 ± 0.04 cm d^{-1}; G15–R15–E5: 0.25 ± 0.04 cm d^{-1}; G15–R15–E15: 0.26 ± 0.04 cm d^{-1}). Within R5,

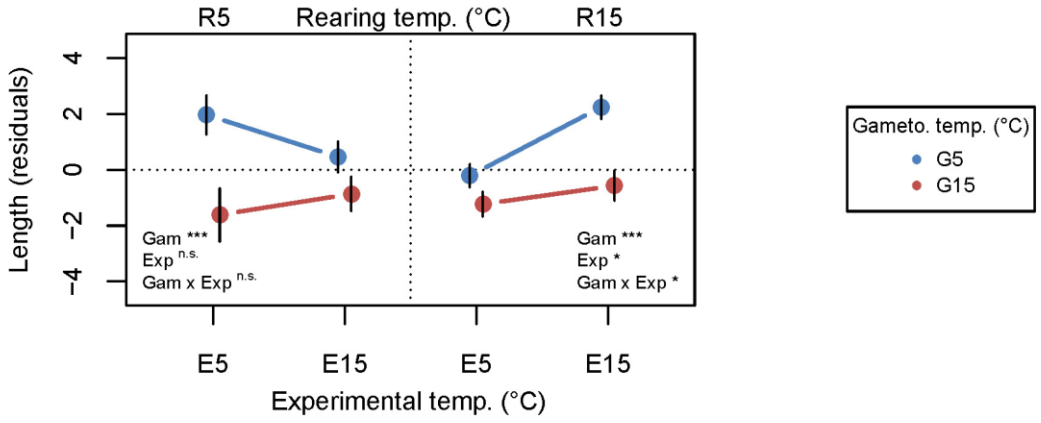

FIGURE 3 | Growth reaction norms of juvenile *Laminaria digitata* sporophytes in experiment 2 to visualize effects of gametogenesis temperature on thermal plasticity of sporophytes. Primary x-axis: experimental temperature, secondary x-axis: rearing temperature, symbol colors: gametogenesis temperature. Residuals of simple linear models of final length as a function of initial length are shown as a growth parameter. The zero-line represents the final length modeled based on initial length, and deviations from the zero-line are interpreted as treatment effects. Mean over all genetic lines ± SE, $n = 20$. Statistical significance of the fixed factors gametogenesis temperature (Gam), experimental temperature (Exp) and their interaction (Gam × Exp) is summarized in the figure; ***$p < 0.001$; *$p < 0.05$; n.s., not significant. For full statistical report see **Table 2**.

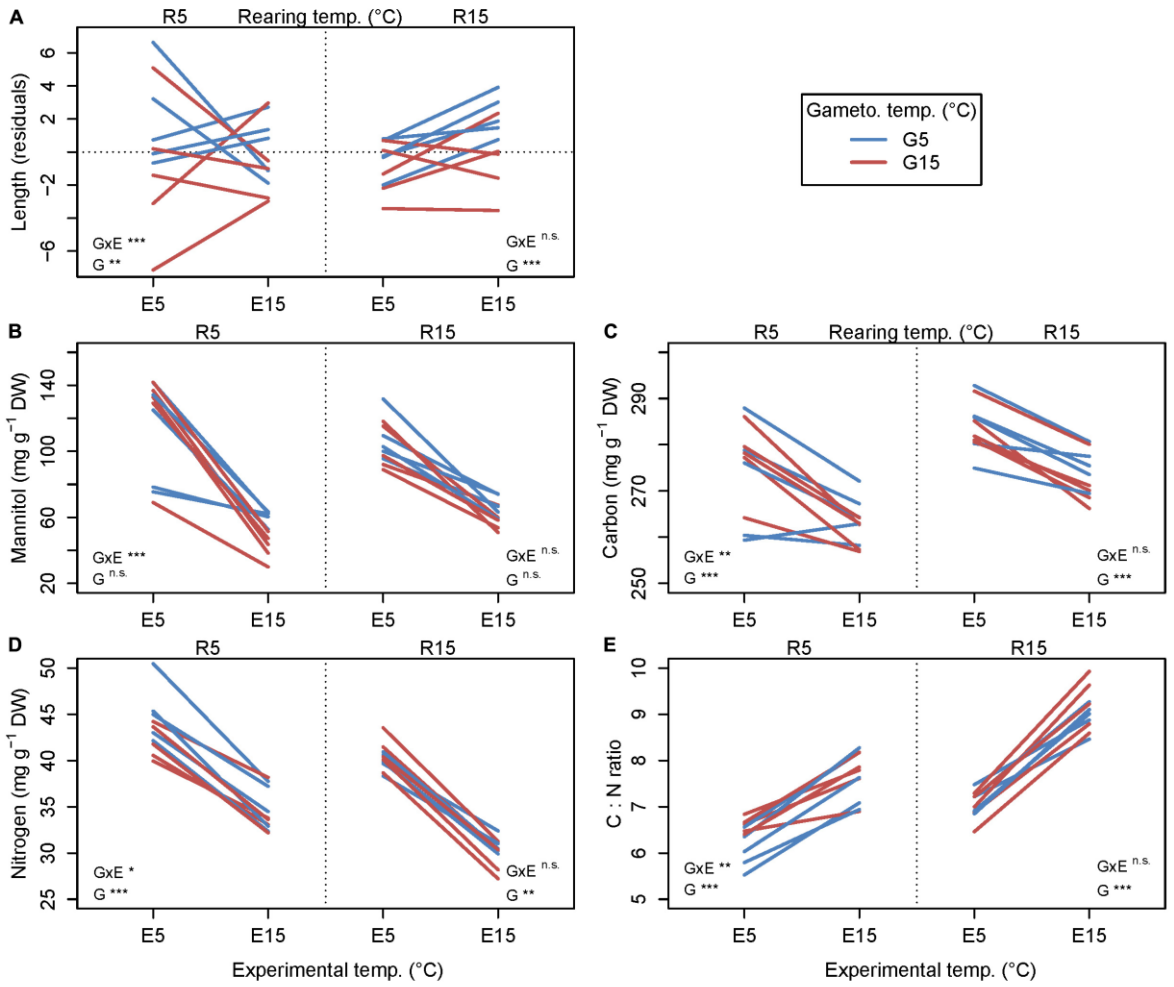

FIGURE 4 | Reaction norms of growth and biochemical parameters for each genetic line of *Laminaria digitata* in experiment 2 to visualize genetic variation for plasticity. Primary x-axis: experimental temperature, secondary x-axis: rearing temperature, slope colors: gametogenesis temperature. All plots show values obtained at the end of the 12-day experiment. **(A)** residual length growth, **(B)** mannitol contents in dry mass, **(C)** total carbon contents in dry mass, **(D)** total nitrogen contents in dry mass, **(E)** carbon to nitrogen mass ratio (C:N ratio). Means over single genetic lines, except for **(B)** means of mean values due to extraction in triplicates, $n = 4$. Statistical significance of the random factors for genetic line × experimental temperature interaction (GxE; random slope) and genetic line (G; random intercept) is summarized in the figure; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; n.s., not significant. For full statistical report see **Table 3**.

growth was highest in the matching gametophyte-sporophyte (gametogenesis-experimental) temperature treatment (G5–R5–E5: 0.38 ± 0.06 cm d^{-1}) compared to the mismatched treatments (G5–R5–E15: 0.36 ± 0.04 cm d^{-1}; G15–R5–E15: 0.34 ± 0.05 cm d^{-1}; G15–R5–E5: 0.31 ± 0.07 cm d^{-1}). Here, the gametogenesis × experimental temperature interaction was marginally non-significant ($p = 0.0859$ after correcting for multiple testing; **Table 2**).

We then tested if genetic lines differed in their thermal plasticity (genetic variation for plasticity) by investigating if model fits significantly improved when including random intercept and slope arguments (**Figure 4** and **Table 3**). A significant random intercept indicates that the magnitude of response to the experimental temperatures differed among genetic lines, whereas a significant random slope indicates that genetic lines differed in the direction and/or expression of their thermal plasticity (genotype × environment interaction; GxE; Saltz et al., 2018). For growth, genetic line (random intercept) was significant at both rearing temperatures (R5: $p = 0.0013$; R15: $p = 0.0002$; **Table 3**), indicating differing magnitudes of response among the five genetic lines within each rearing temperature. Within R5, GxE (random slope) was significant ($p = 0.0001$; **Table 3**), indicating that the genetic lines differed in their plastic growth response to temperature, but only when reared at 5°C. This genetic variation for plasticity is visible as more variance among reaction norms within R5, whereas reaction norm slopes were more similar within R15 (but still differed in magnitude). This increased variance among individuals in R5

TABLE 3 | Results of likelihood ratio tests to determine significance of the random effects "experimental temperature | genetic line" (GxE; random slope) and "1 | genetic line" (G; random intercept) on tested parameters in juvenile *Laminaria digitata* sporophytes in experiment 2.

Parameter	Rearing	Random factor	df	AIC	BIC	logLik	L. ratio	*p*-value
Length	5°C	Exp. temp. | genetic line	10	384.76	408.33	−182.38		
		1 | genetic line	8	398.50	417.35	−191.25	17.74	**0.0001**
		Null	7	406.90	423.40	−196.45	10.40	**0.0013**
	15°C	Exp. temp. | genetic line	10	355.26	355.26	−155.85		
		1 | genetic line	8	329.95	348.81	−156.98	2.26	0.3235
		Null	7	341.52	358.02	−163.76	13.57	**0.0002**
Mannitol	5°C	Exp. temp. | genetic line	12	645.86	674.30	−310.93		
		1 | genetic line	10	665.67	689.37	−322.84	23.81	**<0.0001**
		Null	9	666.27	687.59	−324.13	2.59	0.1070
	15°C	Exp. temp. | genetic line	12	636.42	664.70	−306.21		
		1 | genetic line	10	634.43	658.00	−307.22	2.01	0.3666
		Null	9	632.42	653.63	−307.21	0.01	0.9199
Carbon	5°C	Exp. temp. | genetic line	9	487.92	509.24	−234.96		
		1 | genetic line	7	496.02	512.60	−241.01	12.10	**0.0024**
		Null	6	508.48	522.70	−248.24	14.46	**0.0001**
	15°C	Exp. temp. | genetic line	12	493.15	521.43	−234.57		
		1 | genetic line	10	490.44	514.01	−235.22	1.29	0.5244
		Null	9	500.44	521.65	−241.22	12.00	**0.0005**
Nitrogen	5°C	Exp. temp. | genetic line	12	323.81	352.24	−149.90		
		1 | genetic line	10	327.28	350.97	−153.64	7.47	**0.0239**
		Null	9	356.88	378.20	−169.44	31.60	**<0.0001**
	15°C	Exp. temp. | genetic line	12	255.54	283.82	−115.77		
		1 | genetic line	10	251.56	275.13	−115.78	0.02	0.9894
		Null	9	256.71	277.92	−119.35	7.15	**0.0075**
C:N ratio	5°C	Exp. temp. | genetic line	12	58.44	86.87	−17.22		
		1 | genetic line	10	65.69	89.38	−22.84	11.25	**0.0036**
		Null	9	91.48	112.81	−36.74	27.80	**<0.0001**
	15°C	Exp. temp. | genetic line	12	73.94	102.22	−24.97		
		1 | genetic line	10	74.83	98.40	−27.42	4.89	0.0867
		Null	9	85.47	106.68	−33.73	12.63	**0.0004**
F_v/F_m	5°C	Exp. temp. | genetic line	10	−502.63	−478.94	261.32		
		1 | genetic line	8	−505.93	−486.97	260.96	0.70	0.7036
		Null	7	−507.93	−491.35	260.97	<0.01	0.9644
	15°C	Exp. temp. | genetic line	12	−496.78	−468.50	260.39		
		1 | genetic line	10	−500.06	−476.49	260.03	0.73	0.6947
		Null	9	−502.06	−480.85	260.03	<0.01	0.9437
$rETR_{max}$	5°C	Exp. temp. | genetic line	10	495.61	519.31	−237.81		
		1 | genetic line	8	499.21	518.17	−241.61	7.60	**0.0224**
		Null	7	497.39	513.98	−241.69	0.18	0.6724
	15°C	Exp. temp. | genetic line	12	578.06	606.34	−277.03		
		1 | genetic line	10	574.06	597.63	−277.03	<0.01	0.9997
		Null	9	574.08	595.29	−278.04	2.01	0.1559
I_k	5°C	Exp. temp. | genetic line	12	−72.22	−43.79	48.11		
		1 | genetic line	10	−74.25	−50.56	47.13	1.97	0.3738
		Null	9	−71.69	−50.36	44.84	4.56	**0.0327**
	15°C	Exp. temp. | genetic line	12	−38.42	−10.14	31.21		
		1 | genetic line	10	−42.42	−18.85	31.21	<0.01	1.0000
		Null	9	−44.03	−22.82	31.01	0.39	0.5325
α	5°C	Exp. temp. | genetic line	10	−222.76	−199.06	121.38		
		1 | genetic line	8	−220.78	−201.83	118.39	5.97	0.0504
		Null	7	−222.35	−205.77	118.18	0.43	0.5126
	15°C	Full model did not converge. Comparison only between reduced models.						
		1 | genetic line	8	−222.02	−203.17	119.01		
		Null	7	−224.02	−207.52	119.01	<0.01	0.9629

*Likelihood ratio tests comparing stepwise reduced models of random slopes for experimental temperature * genetic line and random intercepts for genetic line. df, degrees of freedom; AIC, Akaike's An Information Criterion; BIC, Bayesian Information Criterion; logLik, Log-likelihood; L.Ratio, Likelihood-ratio. Significant values ($p < 0.05$) are indicated in bold text. Description of random model arguments: "Exp. temp. | genetic line" describes random slopes over experimental temperatures for each genetic line, i.e., genetic variation for plasticity. "1 | genetic line" describes random intercepts for each genetic line, i.e., different magnitudes of response. Lower AIC and BIC indicate comparatively better model fits. Significant differences are reported for each model in comparison to the model described in the row above.*

TABLE 4 | Results of linear mixed effects models to examine thermal plasticity of biochemical parameters of juvenile *Laminaria digitata* sporophytes in experiment 2.

				Mannitol		Carbon		Nitrogen		C:N ratio	
Rearing	**Parameter**	**numDF**	**denDF**	***F*-value**	***p*-value**	***F*-value**	***p*-value**	***F*-value**	***p*-value**	***F*-value**	***p*-value**
5°C	Initial values	1	70	23.08	**<0.0001**	7.97	**0.0103**	11.04	**0.0025**	42.83	**<0.0001**
	Gametogenesis temp.	1	70	12.81	**0.0012**	14.38	**0.0006**	2.14	0.1962	<0.01	0.9792
	Experimental temp.	1	70	41.11	**<0.0001**	25.18	**<0.0001**	62.69	**<0.0001**	93.63	**<0.0001**
	Gameto × experimental temp.	1	70	21.64	**<0.0001**	19.37	**<0.0001**	12.31	**0.0014**	16.15	**0.0003**
15°C	Initial values	1	69	8.46	**0.0092**	6.70	**0.0176**	15.91	**0.0003**	18.88	**0.0001**
	Gametogenesis temp.	1	69	7.08	**0.0162**	3.16	0.1027	18.77	**0.0001**	7.51	**0.0140**
	Experimental temp.	1	69	79.16	**<0.0001**	47.10	**<0.0001**	1917.75	**<0.0001**	213.31	**<0.0001**
	Gameto × experimental temp.	1	69	0.30	0.6599	3.77	0.0744	16.57	**0.0003**	9.62	**0.0054**

Mannitol content, carbon content, nitrogen content and C:N ratio were tested using initial values as covariates and the interactive effects of gametogenesis temperature and experimental temperature as fixed effects. Genetic line and the interaction between genetic line and experimental temperature were analyzed as random effects. The two rearing temperatures were tested in separate models. numDF and denDF denote numerator and denominator degrees of freedom, respectively. p-values were adjusted for multiple comparisons with FDR corrections. Statistically significant values ($p < 0.05$) are indicated in bold.

might in turn explain the marginally non-significant interaction of gametogenesis and experimental temperature in the mean growth response in R5 (**Figure 3**).

Absolute growth rates show the same pattern of genetic variation for plasticity in R5 (**Supplementary Figure S1**; mind the effect of initial size on growth rates between R5 and R15). Higher growth following G5 was evident in three out of five genetic lines (**Supplementary Figures S1A,C,D**) and in the overall mean response (**Supplementary Figure S1F**). Growth following R5 was either better at 15°C (**Supplementary Figure S1A**) or 5°C (**Supplementary Figure S1B**), and in one case, growth was higher in matching gametogenesis and experimental temperatures (**Supplementary Figure S1C**).

Biochemistry

All biochemical parameters were significantly influenced by gametogenesis temperature, experimental temperature, and interactive effects of gametogenesis × experimental temperature (**Figures 4B–E** and **Table 4**; for means over all genetic lines see **Supplementary Figure S2**). For all parameters, the main effect of experimental temperature was most distinct, indicating fast thermal acclimation capacity of biochemical pathways. Effects of gametogenesis temperature were weak. Overall, there were higher carbon, nitrogen and mannitol contents at E5 than at E15 (**Figures 4B–E** and **Table 4**). Additionally, genetic variation for plasticity (i.e., variation in reaction norm slopes) was significant for all biochemical parameters, but only following rearing at 5°C (**Table 3**).

Mean mannitol content (**Figure 4B**) was significantly higher at E5 than at E15 for both rearing temperatures (R5: 128% higher at E5, $p < 0.0001$; R15: 68% higher, $p < 0.0001$; **Table 4**) irrespective of gametogenesis temperature. For mannitol, response magnitude (random intercept) did not differ significantly among genetic lines in either rearing temperature, but response plasticity (random slope) differed significantly among genetic lines in R5 ($p < 0.0001$; **Table 3**). The significant interaction of gametogenesis and experimental temperatures in R5 ($p < 0.0001$; **Table 4**) was likely driven by the reaction norms of two genetic lines in G5–R5 that had shallower slopes than the rest (**Figure 4B**; see also the crossing mean reaction norms in **Supplementary Figure S2A**).

A similar pattern arose for carbon content (**Figure 4C**), where values were significantly higher at E5 than at E15 for both rearing temperatures (R5: 4.5% higher at E5, $p < 0.0001$; R15: 4.0% higher, $p < 0.0001$; **Table 4**) irrespective of gametogenesis temperature. For carbon, response magnitude differed significantly among genetic lines in both rearing temperatures (R5: $p = 0.0001$; R15: $p = 0.0005$; **Table 3**), but response plasticity was only significant within R5 ($p = 0.0024$; **Table 3**). This again indicates genetic variation for plasticity only following rearing at 5°C, with two genetic lines in G5–R5 having comparatively shallow (and even opposite) slopes compared to the other lines (**Figure 4C**). The significant interaction of gametogenesis × experimental temperature in R5 ($p < 0.0001$; **Table 4**) is visible as crossing mean reaction norms of G5–R5 and G15–R5, due to lower mean carbon contents in G5–R5–E5 (**Figure 4C**; see also **Supplementary Figure S2B**).

Nitrogen contents (**Figure 4D**) were also primarily influenced by experimental temperature, with significantly higher values at E5 than at E15 for both rearing temperatures (R5: 26% higher at E5, $p < 0.0001$; R15: 34% higher, $p < 0.0001$; **Table 4**) irrespective of gametogenesis temperature. Again, response magnitude differed significantly among genetic lines in both rearing temperatures (R5: $p < 0.0001$; R15: $p = 0.0075$; **Table 3**), but response plasticity was only significant within R5 ($p = 0.0239$; **Table 3**). The interaction between gametogenesis and experimental temperatures was significant for both rearing temperatures (R5: $p = 0.0014$; R15: $p = 0.0003$; **Table 4**), seen as slight differences in overall reaction norm slopes between G5 and G15 (**Supplementary Figure S2C**).

C:N ratios (**Figure 4E**) generally followed a reverse pattern to that of nitrogen content because relative differences in carbon content among treatments were of much smaller magnitude than differences in nitrogen contents. C:N ratios were primarily influenced by experimental temperature, with significantly higher values at E15 than at E5 for both rearing temperatures (R5: 21% higher at E15, $p < 0.0001$; R15: 29% higher, $p < 0.0001$; **Table 4**) irrespective of gametogenesis temperature. Response magnitude differed significantly among genetic lines in both rearing temperatures (R5: $p < 0.0001$; R15: $p = 0.0004$; **Table 3**), but response plasticity was only significant within R5 ($p = 0.0036$; **Table 3**). The significant two-way interactions

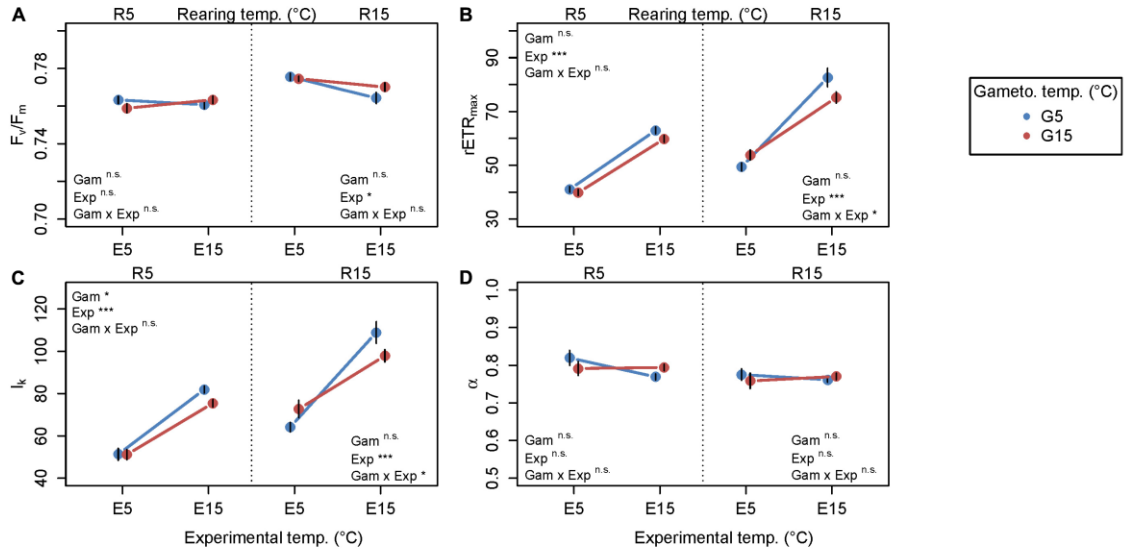

FIGURE 5 | Reaction norms of fluorometric parameters averaged over all five genetic lines of juvenile *Laminaria digitata* sporophytes in experiment 2. Primary x-axis: experimental temperature, secondary x-axis: rearing temperature, symbol colors: gametogenesis temperature. All plots show values obtained at the end of the 12-day experiment. **(A)** Optimum quantum yield F_v/F_m. **(B)** Maximum relative electron transport rate $rETR_{max}$. **(C)** Saturation irradiance I_k (μmol photons m^{-2} s^{-1}). **(D)** Photosynthetic efficiency α (rETR/μmol photons m^{-2} s^{-1}). Mean values ± SE over all genetic lines, $n = 20$. Statistical significance of the fixed factors gametogenesis temperature (Gam), experimental temperature (Exp), and their interaction (Gam × Exp) is summarized in the figure; ***$p < 0.001$; *$p < 0.05$; n.s., not significant. For full statistical report see **Table 5**.

TABLE 5 | Results of linear mixed effects models to examine thermal plasticity of fluorometric parameters of juvenile *Laminaria digitata* sporophytes in experiment 2.

				F_v/F_m		$rETR_{max}$		I_k		α	
Rearing	**Parameter**	**numDF**	**denDF**	***F*-value**	***p*-value**	***F*-value**	***p*-value**	***F*-value**	***p*-value**	***F*-value**	***p*-value**
5°C	Initial values	1	70	7.40	**0.0132**	0.14	0.7472	1.16	0.3381	1.52	0.2744
	Gametogenesis temp.	1	70	0.99	0.3724	3.66	0.0859	6.53	**0.0198**	2.02	0.2055
	Experimental temp.	1	70	0.04	0.8662	90.14	**<0.0001**	79.38	**<0.0001**	0.69	0.4591
	Gameto × experimental temp.	1	70	3.16	0.1090	0.61	0.4783	1.49	0.2744	4.01	0.0735
15°C	Initial values	1	69	1.85	0.2164	11.67	**0.0022**	5.69	**0.0273**	0.21	0.7056
	Gametogenesis temp.	1	69	0.09	0.7791	0.66	0.4841	0.20	0.7056	0.16	0.7271
	Experimental temp.	1	69	6.92	**0.0169**	185.16	**<0.0001**	140.12	**<0.0001**	<0.01	0.9806
	Gameto × experimental temp.	1	69	2.53	0.1451	6.85	**0.0169**	7.09	**0.0162**	0.85	0.4249

Optimum quantum yield F_v/F_m, maximum relative electron transport rate $rETR_{max}$, saturation irradiance I_k and photosynthetic efficiency α were tested using initial values as covariates and the interactive effects of gametogenesis temperature and experimental temperature as fixed effects. Genetic line and the interaction between genetic line and experimental temperature were analyzed as random effects. The two rearing temperatures were tested in separate models. Non-normality of residuals for I_k was treated by log-transformation (Underwood, 1997). numDF and denDF denote numerator and denominator degrees of freedom, respectively. p-values were adjusted for multiple comparisons with FDR corrections. Statistically significant values ($p < 0.05$) are indicated in bold.

of gametogenesis × experimental temperature in both rearing temperatures (R5: $p = 0.0003$; R15: $p = 0.0054$; **Table 4**) are visible as slightly different (non-parallel) overall reaction norm slopes between G5 and G15 (**Supplementary Figure S2D**).

Fluorometry

Fluorometry results are presented as mean reaction norms over all genetic lines (**Figure 5**), as genetic variation for plasticity among genetic lines was not significant for most fluorometric parameters (**Table 3**; reaction norms of single genetic lines are presented in **Supplementary Figure S3**). Similarly to biochemistry, fluorometric characteristics were predominantly influenced by experimental temperature, with only occasional significant effects of gametogenesis temperature or interactive effects (**Figure 5** and **Table 5**). For optimum quantum yield (F_v/F_m; **Figure 5A**), only experimental temperature had a significant effect within R15 ($p = 0.0169$; **Table 5**), in which F_v/F_m was significantly higher at E5 (0.775 ± 0.007, mean ± SD) than at E15 (0.767 ± 0.011, mean ± SD). F_v/F_m in juvenile sporophytes thereby showed a different response than the field material (experiment 1, **Figure 2**), in which values were significantly higher at 15°C across seasons. All samples expressed high F_v/F_m values (> 0.7), indicating good sample health over the course of the experiment. For F_v/F_m, response magnitude and plasticity did not differ significantly among genetic lines (**Supplementary Figure S3**, **Table 3**). Maximum relative electron transport rate

($rETR_{max}$; **Figure 5B**) and saturation irradiance (I_k; **Figure 5C**) responded similarly. Both were most strongly influenced by experimental temperature ($p < 0.0001$ for both parameters in R5 and R15; **Table 5**), with values at E15 about 30–70% higher than at E5. The significant gametogenesis × experimental temperature interaction within R15 for both $rETR_{max}$ ($p = 0.0169$; **Table 5**) and I_k ($p = 0.0162$; **Table 5**) is shown by the steeper slopes of G5 compared to G15, leading to the highest values in G5–R15–E15 (**Figures 5B,C**). For $rETR_{max}$, response plasticity (random slope) significantly differed among genetic lines at R5 ($p = 0.0224$, **Table 3**, **Supplementary Figure S3B**), while for I_k, response magnitude (random intercept) significantly differed at R5 ($p = 0.0327$, **Table 3** and **Supplementary Figure S3C**). Photosynthetic efficiency (α; **Figure 5D**) was not significantly affected by any of the tested factors (**Tables 3**, **5**). For all fluorometric parameters, standard deviations among the four replicates within the genetic lines were comparable to the extent of variation among genetic lines (data not shown), which likely caused non-significance of random slopes and random intercepts, despite visual differences among mean reaction norms (**Supplementary Figure S3**).

DISCUSSION

Our study was designed to test for effects of temperature during germline development and ontogeny on thermal plasticity of juvenile kelp sporophytes. Our major findings suggest that early exposure to cold temperature promotes the development of juvenile *Laminaria digitata* sporophytes. This contrasts our hypothesis that matching temperatures across ontogeny, specifically in the context of climate warming, should benefit kelp trait performance. Most importantly, gametogenesis and recruitment at 5°C promoted faster growth of 3–4 month old offspring sporophytes across all thermal environments, indicating persistent carry-over effects across ontogeny. Interestingly, reaction norm slopes (i.e., thermal plasticity) differed significantly among five genetic lines, but this variation for plasticity only became apparent in sporophytes reared at 5°C. Following rearing at cold temperature, there was no overall growth benefit of either 5 or 15°C among genetic lines due to high variation in plasticity. Such effects of temperature history and genotype may explain slight differences in previous reports of *L. digitata*'s temperature characteristics, where growth was either the same at 5 and 15°C (Bolton and Lüning, 1982) or better at 15°C (tom Dieck, 1992; Franke, 2019). We also detected interactive effects of gametogenesis and experimental temperatures on thermal plasticity of growth, biochemical and fluorometric characteristics of juvenile sporophytes, but these effects were weak, providing further evidence that potential cross-generational effects may not be a panacea in the face of climate change (Byrne et al., 2020). Biochemical and fluorometric parameters were generally highly plastic and mainly responded to experimental temperatures, indicating fast acclimation responses. For example, mannitol, carbon and nitrogen storage were significantly higher at 5°C compared to 15°C, while photosynthetic capacity increased at 15°C. Genetic variation for plasticity was also only evident following cold rearing for biochemical parameters. Taken together, trait performance was highest following either cold gametogenesis and recruitment, cold offspring rearing, or cold experimental temperatures, suggesting potential consequences for the persistence of cold-temperate kelp populations under climate warming.

Seasonal Growth of Wild *L. digitata* Sporophytes

In experiment 1, meristem tissue from wild sporophytes generally grew faster at 15°C compared to 5°C or at the same rate (May). Thus, our field-collected sporophytes responded similarly to published evidence from juvenile laboratory-grown *L. digitata* sporophytes, with either no difference in growth at 5 and 15°C (Bolton and Lüning, 1982) or a clear benefit of 15°C (tom Dieck, 1992; Franke, 2019). Higher growth at 15°C was especially striking in late winter following cold *in situ* temperatures. High growth rates in late winter can be explained by the start of the fast growing season for *L. digitata* (Perez, 1971; Kain, 1979; Gomez and Lüning, 2001), which is mediated by an endogenous circannual rhythmicity and the transition from short to long daylengths (Schaffelke and Lüning, 1994). Therefore, although conditions in the field modified the magnitude of temperature plasticity of *Laminaria digitata* adult sporophytes throughout the year, the general response pattern was stable, with warm temperatures generally favoring growth. However, the observation of higher growth of a few individuals at 5°C in May indicates that this pattern might not be true for all genotypes, especially when taking into account their environmental history.

Gametogenesis at Cold Temperature Promotes Offspring Sporophyte Growth

Growth responses of 3–4 month old juvenile sporophytes were not uniform, but differed depending on genetic line and their temperature history across generations and ontogeny (experiment 2). When comparing mean growth over G5 and G15, an experimental temperature of 15°C was beneficial (following R15) or the same (following R5) as 5°C for growth over a 12–day period, as shown in other studies (Bolton and Lüning, 1982; tom Dieck, 1992; Franke, 2019). However, in our study, growth responses differed significantly among temperature history treatments. Our initial hypothesis that matching gametogenesis/recruitment and experimental temperatures would improve trait performance was not confirmed. Rather, a gametogenesis temperature of 5°C increased sporophyte growth in the mismatching G5–R15–E15 treatment.

Most importantly, the gametogenesis treatment of 5°C generally improved growth of juvenile sporophytes in both rearing temperatures (**Figure 3**), and this was most strongly expressed in the matching rearing-experimental environments (G5–R5–E5 and G5–R15–E15). This indicates a beneficial effect of a cold environment during gametogenesis and/or recruitment which persisted in juvenile sporophyte offspring. Several potential underlying mechanisms may be involved: exposure of newly recruited sporophytes to the cold parental environment might have induced beneficial carry-over effects within the sporophyte generation (Palmer et al., 2012; Byrne et al., 2020). For instance, a stimulating effect of 5°C on assimilative

processes (see carbon and nitrogen storage, **Figure 4**) of newly recruited sporophytes might have mediated faster growth in 3–4 month-old sporophytes. Potentially, epigenetic mechanisms such as DNA methylation (Qu et al., 2013; Fan et al., 2020) or histone modification (Bourdareau, 2018; Pearson et al., 2019) initiated during early ontogeny may facilitate changes in gene transcription during later life. However, this emerging field of research does not yet provide substantial evidence for these processes in kelps. Alternatively, the mediating factor could be exposure of the parental germline to cold temperature. In this respect, our results could reflect a "silver spoon" parental effect (Uller et al., 2013; Baker et al., 2019; Germain et al., 2019). "Silver spoon" effects describe an advantage for individuals born to parents with access to abundant resources, which benefits offspring through, e.g., maternal provisioning. Mechanisms facilitating resource-based "silver spoon" effects in kelps could potentially include energy transfer between haploid and diploid generations, for example via gamete lipid content (Brzezinski et al., 1993). Neutral lipids are the major form of carbon storage in kelp meiospores and gametes (eggs and sperm), and are therefore crucial for kelp reproduction (Brzezinski et al., 1993; Reed et al., 1999). A higher lipid concentration in germ cells could give zygotes an energetic advantage across generations, but temperature effects on lipid storage are as yet unknown.

Interestingly, in *L. digitata*, gametogenesis is faster at 15°C than 5°C, but recruitment is twice as high at 5°C (Martins et al., 2017). This might be due to the prolonged vegetative growth of gametophytes at 5°C compared to 15°C (pers. obs.), which may result in more female cells per gametophyte. As all female cells may develop into oogonia, this was previously discussed as a potentially adaptive response increasing recruitment at non-optimal reproductive conditions (Bolton and Levitt, 1985). Our study reveals that in addition to higher recruitment at cold temperature (Martins et al., 2017), gametogenesis and recruitment at cold temperature seems to be a prerequisite for enhanced juvenile sporophyte growth of *L. digitata*. This effect was evident under constant long daylengths, which suppress the free-running growth rhythm of *L. digitata* (Schaffelke and Lüning, 1994). Whether photoperiod or endogenous circannual rhythms also affect thermal plasticity of *L. digitata* is yet unknown.

High Acclimation Potential of Biochemical and Fluorometric Parameters

Biochemical contents (mannitol, carbon, nitrogen) of juvenile sporophytes were mostly influenced by acclimation responses to experimental temperatures, favoring 5 over 15°C (**Figure 4** and **Table 4**), and only showed weak interactive effects between gametogenesis and experimental temperature. Therefore, effects of temperature during gametogenesis and recruitment on biochemical trait plasticity might be negligible in the wild compared to the high and fast acclimation capacity in response to the immediate environment. Increased carbon and mannitol contents at 5°C indicated more efficient carbon assimilation processes at cold temperature, despite possibly lower photosynthetic capacity (**Figure 5**). This contrasts the naturally occurring peaks in carbon storage in late summer when growth is minimal (Haug and Jensen, 1954; Schiener et al., 2015). Naturally, a temperature of 5°C would rather co-occur with short daylengths at our study location of Helgoland. Therefore, the pattern of high growth and high carbon storage at 5°C might be a product of experimental long days in our experiment, which may stimulate carbon storage accumulation (in *L. hyperborea*; Schaffelke, 1995).

Fluorometric results indicated photoacclimative responses to experimental temperature (**Figure 5** and **Table 5**), but in contrast to biochemical contents, the maximum relative electron transport rate ($rETR_{max}$) and saturation irradiance (I_k) were promoted at warm temperature. Photosynthetic rates usually increase at higher, sublethal temperatures (Delebecq et al., 2016; Burdett et al., 2019), with photosynthetic thermal optima often exceeding thermal growth optima (Eggert and Wiencke, 2000; Graiff et al., 2015; Fernández et al., 2020). In E5, maximum relative electron transport rate $rETR_{max}$ was close to the irradiance conditions in our experiment (30–40 μmol photons m^{-2} s^{-1}), while $rETR_{max}$ was higher at E15. Therefore, faster metabolism and higher energy demands at 15°C might have reduced carbon and mannitol storage compared to 5°C.

Nitrogen contents were highest in the matching G5–R5–E5 thermal history and lowest in G15–R15–E15 (**Figure 4** and **Table 4**). Due to the high amount of nitrogen storage in all E5 treatments, C:N ratios were approximating the Redfield ratio of 106C:16N (= 6.625). Atkinson and Smith (1983) described this value as the lower limit, which is only attainable in strongly nitrogen-enriched environments, such as present in our PES cultivation medium (Sarker et al., 2013), and which is characteristic of rapid growth phases in seaweeds (Niell, 1976). As all samples were treated with the same nutrient enrichment and all grew well, the high nitrogen contents only in E5 were probably not the cause of fast growth. From an ecological standpoint, high internal nitrogen concentrations at cold temperature might indicate a seasonal pattern, as *L. digitata*'s growth period starts in late winter to spring, when the nutrient content of seawater is high (Davison et al., 1984; Wiltshire et al., 2015). Nitrate reductase (NR) activity peaks in spring in a Scottish *L. digitata* population (Davison et al., 1984), despite a potentially wide temperature range for kelp NR activity (Fernández et al., 2020), suggesting that the year-round uptake potential might be controlled by interactive seasonal patterns of nitrate availability, NR activity, temperature and irradiance, among others (Young et al., 2007a,b). However, nutrient uptake across temperature or seasonal gradients is rarely studied for seaweeds (Roleda and Hurd, 2019).

Genetic Variation for Plasticity

Variation for plasticity among genotypes offers a target for selection in a warming environment if the variation of traits is heritable (Chevin et al., 2010; Munday et al., 2017, 2019), and is an important component of the adaptive capacity of populations and species. Already during recruitment of juvenile sporophytes for experiment 2, we observed that one out of ten genetic lines recruited more sporophytes at 15°C than at 5°C

(**Supplementary Figure S4** and **Supplementary Table S3**). This was a first indication that the tested genetic lines might express differential plasticity and differing temperature preferences. This was also indicated in experiment 1, where in May, a few individuals had higher growth at 5°C than 15°C (**Supplementary Table S1**), whereas mean growth of all 20 individuals did not differ between 5 and 15°C (**Figure 2**).

In experiment 2, thermal reaction norms of growth and biochemical characteristics varied in magnitude (intercept) and plasticity (slope) among the five genetic lines (**Figure 4** and **Table 3**), indicating that genetic lines differed in their expression of genotype × environment interactions (see also Galloway, 2001; Suter and Widmer, 2013). Interestingly, while the magnitude of response varied significantly in both rearing temperatures, only the 5°C rearing environment was associated with significant variation for plasticity among the five genetic lines (**Figure 4** and **Table 3**). Therefore, temperature history modulated the extent of plastic responses, in that cold rearing generally induced higher thermal plasticity in growth and biochemical responses than warm rearing. Variation for thermal plasticity was most obvious for growth, where each genetic line responded differently (**Figure 4** and **Supplementary Figure S1**). This variation in thermal responses across genetic lines seemed to outweigh the effects of temperature history treatments (gametogenesis/recruitment and rearing) on response plasticity toward the experimental temperatures (**Figures 3**, **4**).

Our results show that thermal responses were highly plastic, and that better growth of *L. digitata* at 15°C might not be universal, but differs depending on genetic background and thermal history. Furthermore, thermal carry-over effects from the rearing treatment possibly modulated the extent of genotype × environment interactions on trait responses. As phenotypic trait variation is an important target for natural selection (Chevin et al., 2010; Kelly, 2019), this could have important consequences for the adaptive capacity of *Laminaria digitata* populations during ocean warming. For instance, if phenotypic variation of adaptive traits among genotypes is lower in sporophytes recruited at warmer temperature, then selection is less able to remove maladaptations from a population. This in turn would decrease the strength of directional selection (Ghalambor et al., 2015), and therefore could reduce the adaptive capacity of the population in a negative feedback loop under increasingly warm temperature.

The Importance of Cold Seasons

We hypothesized that trait performance should increase in matching parent gametophyte and offspring sporophyte environments. However, interactive temperature effects played a minor role in shaping the reaction norms of juvenile *L. digitata* sporophytes. Rather, gametogenesis and recruitment at 5°C benefited mean growth of juvenile sporophytes, sporophyte rearing at 5°C enabled high thermal plasticity, and carbon and nitrogen contents significantly increased in the 5°C experimental treatment. In this study, we add to previous evidence of higher recruitment at a cold temperature (5°C) compared to a warm temperature (15°C) for *L. digitata* (Martins et al., 2017), and show a general disadvantage of early ontogeny at 15°C compared to 5°C for juvenile *L. digitata* sporophytes.

Across *L. digitata*'s European distribution, temperature regimes differ substantially. While mean monthly SST vary between 0 and 7°C over the year in Kongsfjorden, Spitsbergen (Müller et al., 2009; Bartsch et al., 2016), mean monthly SST range between 7 and 18°C at the southern distribution limit in Quiberon, France (Oppliger et al., 2014). *L. digitata* sporophytes become fertile mostly from early summer to late autumn along their latitudinal distribution (Sjøtun and Schoschina, 2002; Bartsch et al., 2008, 2013; Olischläger and Wiencke, 2013). According to our results, northern to Arctic populations may be favored by gametogenesis, recruitment and growth of early sporophytes during summer to autumn conditions (Sjøtun and Schoschina, 2002) at cold temperature (5°C) and long daylength. Further, these conditions increase sporophyte recruitment compared to warmer temperatures and/or a short photoperiod (Martins et al., 2017). Conversely, summer temperatures at the warm distribution limit (≥18°C) such as at our study location Helgoland (**Figure 2**; Wiltshire et al., 2008; Bartsch et al., 2013) or Quiberon (Oppliger et al., 2014), may induce vegetative persistence of gametophytes after summer spore release (tom Dieck, 1992; Martins et al., 2017). We provide evidence that recruitment at the end of southern summers, when decreasing temperatures allow gametogenesis (e.g., 15°C; Martins et al., 2017), might lead to reduced growth and reduced thermal plasticity of juvenile sporophytes. The comparative benefit of early ontogeny at 5°C demonstrated in this study will be achievable throughout the year at northern locations, but only in winter to spring at southern locations. Subsequent investigations into optimal conditions for recruitment and thermal plasticity are needed, as temperature and daylength may shape responses interactively (Schaffelke and Lüning, 1994; Martins et al., 2017), and as populations might differ in their thermal plasticity (King et al., 2018, 2019).

With a predicted warming of the North Atlantic by 1–3°C (Müller et al., 2009; Schrum et al., 2016), southern *L. digitata* populations are threatened by rising maximum temperatures and marine heatwaves (Raybaud et al., 2013; Assis et al., 2018). This is especially true for populations where sporophytes already encounter temperatures at their upper tolerance limit (e.g., Helgoland, North Sea, Bartsch et al., 2013; Quiberon, Brittany, Oppliger et al., 2014), which affect traits such as reproduction, photosynthesis, growth and mortality (Bartsch et al., 2013; Burdett et al., 2019; Nepper-Davidsen et al., 2019). As suggested by our data, rising temperatures might not only have immediate detrimental effects such as local extinctions of populations due to marine heatwaves (reviews by Straub et al., 2019; Smale, 2020). Reduced growth and thermal plasticity induced by warming during early ontogeny might additionally lead to an overall weakened performance of southern *L. digitata* populations.

DATA AVAILABILITY STATEMENT

The raw data and R code generated during this study are available in the PANGAEA database (https://doi.org/10.1594/PANGAEA.917810).

AUTHOR CONTRIBUTIONS

DL, LS, and IB planned and designed the experiments. DL conducted the experiments. ND performed PAM measurements and rapid light curve fits. DL and LS analyzed the data. IB supervised the project. DL, IB, LS, ND, and KV contributed to the data interpretation and discussion. DL wrote the manuscript, which was reviewed, revised, and approved by all authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00456/full#supplementary-material

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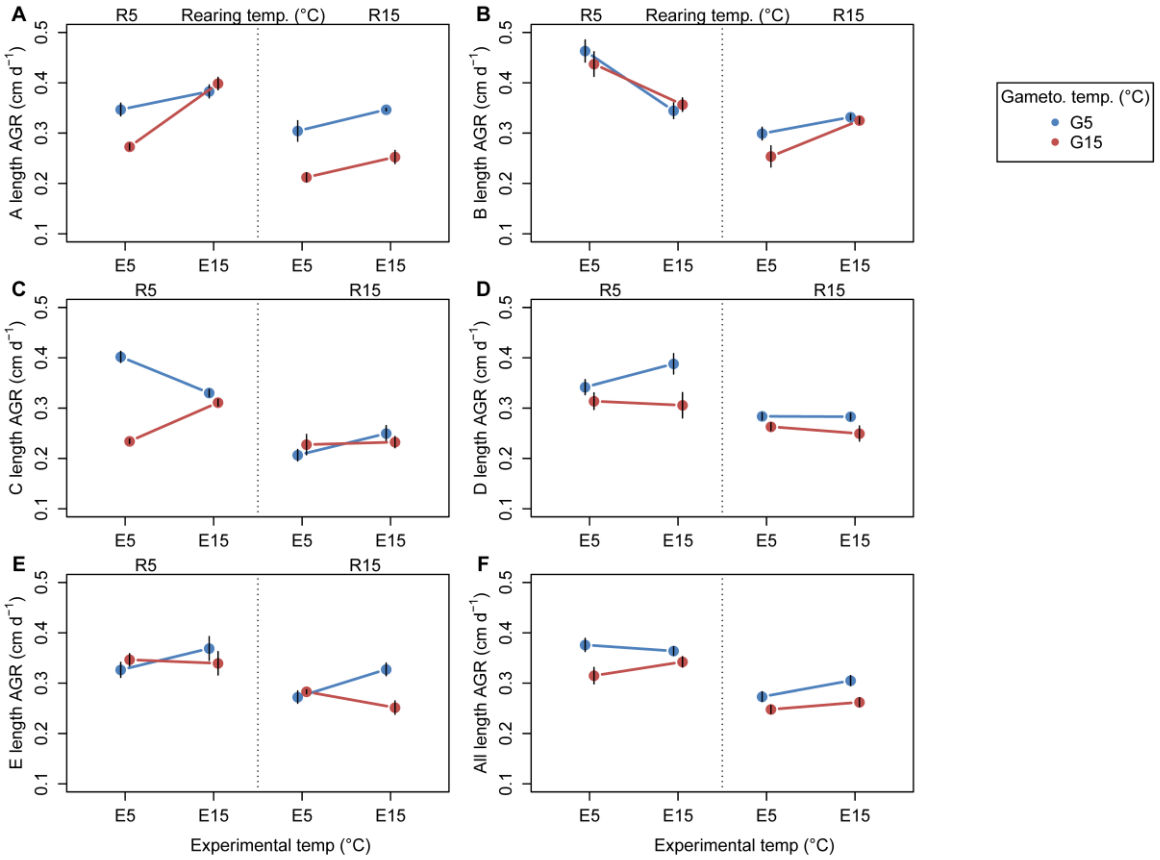
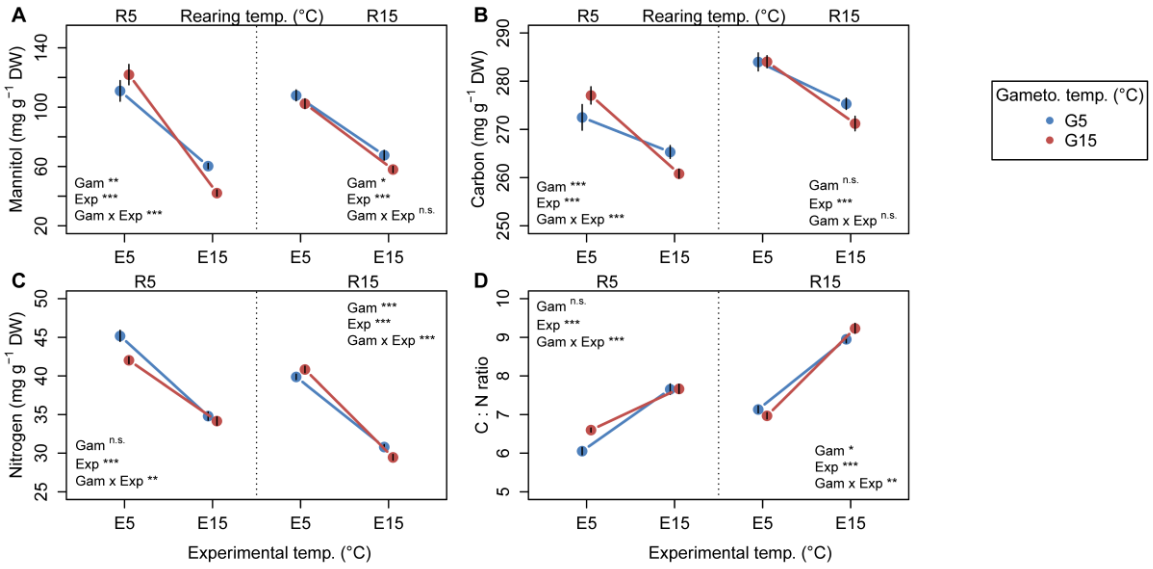
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

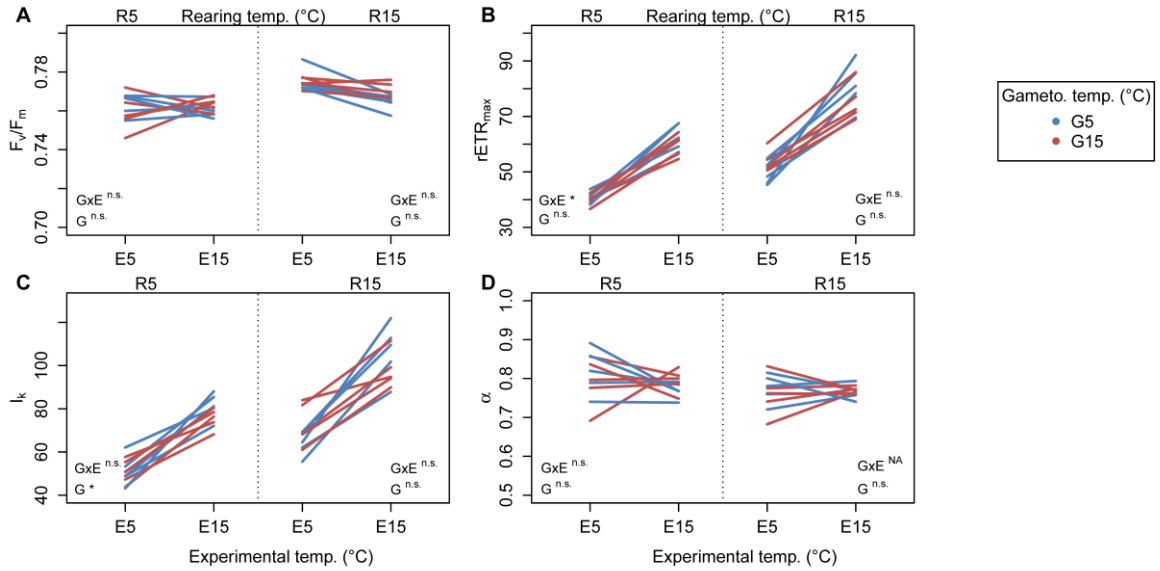
SUPPLEMENTARY MATERIAL



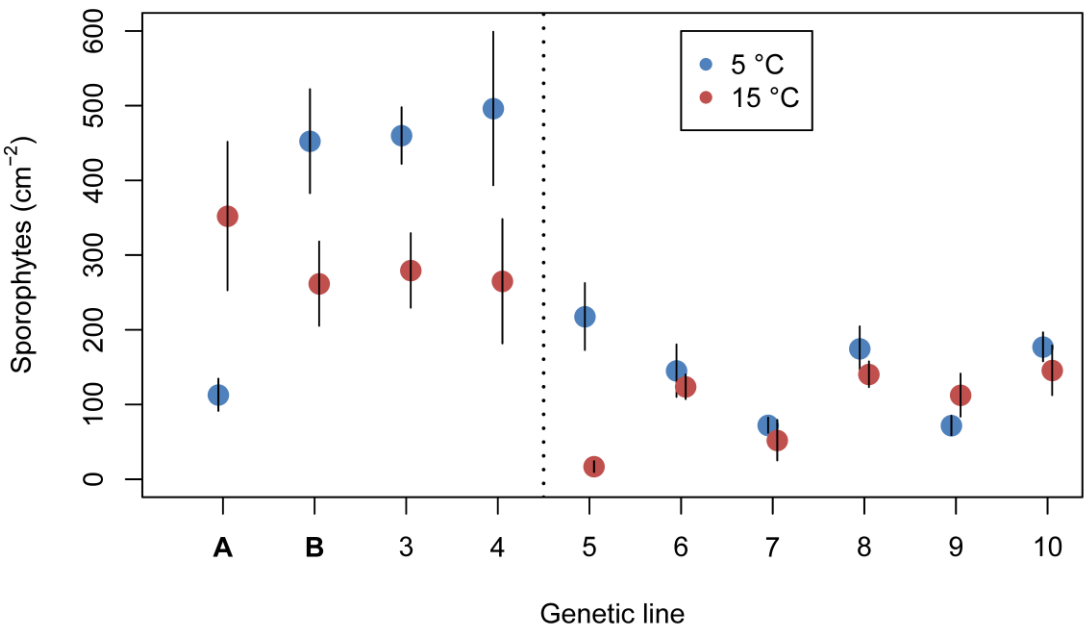
Supplementary Figure S1 Temperature reaction norms of length growth rates of single genetic lines (A – E) and averaged over all genetic lines (F) of *Laminaria digitata* during experiment 2. Primary x-axis: experimental temperature, secondary x-axis: rearing temperature, symbol colours: gametogenesis temperature. Mean values ± SE, $n = 4$ for five genetic lines, $n = 20$ for all genetic lines.



Supplementary Figure S2 Temperature reaction norms of biochemical parameters averaged over all five genetic lines of juvenile *Laminaria digitata* sporophytes in experiment 2. Primary x-axis: experimental temperature, secondary x-axis: rearing temperature, symbol colours: gametogenesis temperature. All plots show values obtained at the end of the 12-day experiment. (A) Mannitol contents in dry mass. (B) Total carbon contents in dry mass. (C) Total nitrogen contents in dry mass. (D) Carbon to nitrogen mass ratio. Mean values ± SE over all genetic lines, except for (A) means of mean values due to extraction in triplicates, n = 20. Statistical significance of the fixed factors gametogenesis temperature (Gam), experimental temperature (Exp) and their interaction (Gam x Exp) is summarized in the Figure; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., not significant. For full statistical report see **Table 4**.



Supplementary Figure S3 Temperature reaction norms of fluorometric parameters for each genetic line of *Laminaria digitata* in experiment 2 to visualize genetic variation for plasticity. Primary x-axis: experimental temperature, secondary x-axis: rearing temperature, slope colours: gametogenesis temperature. All plots show values obtained at the end of the 12-day experiment. (A) Optimum quantum yield F_v/F_m. (B) Maximum relative electron transport rate $rETR_{max}$. (C) Saturation irradiance I_k (µmol photons m^{-2} s^{-1}). (D) Photosynthetic efficiency α (rETR / µmol photons m^{-2} s^{-1}). Means over single genetic lines, $n = 4$. Statistical significance of the random factors for genetic line x experimental temperature interaction (GxE; random slope) and genetic line (G; random intercept) is summarized in the Figure; *, $p < 0.05$; n.s., not significant; NA, not available. For full statistical report see **Table 3**.



Supplementary Figure S4 Sporophyte recruitment of individual genetic lines of *Laminaria digitata* following gametogenesis after 26 days at 5°C and 15°C (microscopic counts, n = 4, mean ± SE). Recruitment was saturated after 19 days at 15°C and after 26 days at 5°C. Only genetic lines A and B were present throughout experiment 2 (indicated in bold); genetic lines C–E were not assessed during gametogenesis and recruitment. Spore densities differed between genetic lines at time of inoculation. Between genetic lines A, B, 3 and 4, gametophyte densities did not differ significantly after meiospore germination on day 4 (marked by dashed line). Note the majority of sporophyte recruitment at 15°C only in genetic line A. See **Supplementary Table S3** for statistical analysis.

Supplementary Table S1 Absolute growth rates of individual meristematic *Laminaria digitata* discs over 14 days at 5°C and 15°C in experiment 1.

	February		May		July	
	5°C	15°C	5°C	15°C	5°C	15°C
Individuals	0.378	1.303	0.316	0.327	0.173	0.272
	0.285	0.513	**0.176**	**0.113**	0.232	0.412
	0.362	0.994	**0.165**	**0.138**	0.336	0.377
	0.729	0.807	0.124	0.357	0.342	0.364
	0.707	1.143	**0.365**	**0.332**	0.277	0.577
	0.557	1.269	**0.114**	**0.113**	0.164	0.391
	0.188	0.381	0.261	0.572	0.248	0.476
	0.285	0.382	**0.272**	**0.236**	0.291	0.427
	0.274	0.727	0.106	0.169	0.153	0.152
	0.439	1.241	0.221	0.457	0.165	0.237
	0.227	0.461	0.204	0.253	0.081	0.106
	0.480	1.412	**0.333**	**0.226**	0.149	0.176
	0.212	0.364	**0.199**	**0.116**	0.167	0.210
	0.711	0.982	**0.183**	**0.172**	0.133	0.223
	0.312	0.660	0.322	0.539	0.189	0.370
	0.257	1.097	0.184	0.223	0.168	0.406
	0.202	0.364	0.317	0.515	0.203	0.286
	0.544	0.758	**0.278**	**0.108**	0.211	0.321
	0.233	0.579	0.338	0.386	0.228	0.406
	0.411	0.796	0.335	0.382	0.212	0.334

For each month, growth rates ($cm^2\ d^{-1}$) at 5°C and 15°C each correspond to one meristem disc of the same wild sporophyte individual. $N = 60$. All discs had the same initial size (Ø 24 mm). Individuals growing faster at 5°C than at 15°C are highlighted in bold text.

Supplementary Table S2 Initial values for growth, biochemical and fluorometric parameters of juvenile *Laminaria digitata* sporophytes before experiment 2.

Initial values	R5		R15	
	G5	G15	G5	G15
Length per sporophyte (cm)	11.1 ± 1.0	11.1 ± 0.9	7.1 ± 1.5	6.9 ± 1.2
Mannitol (mg g^{-1} dm)	92.7 ± 16.0	73.6 ± 17.6	52.7 ± 4.9	52.1 ± 7.6
Carbon (mg g^{-1} dm)	268.9 ± 8.8	255.9 ± 9.0	272.5 ± 3.2	271.8 ± 1.7
Nitrogen (mg g^{-1} dm)	25.1 ± 1.2	21.8 ± 0.6	24.7 ± 1.2	24.1 ± 2.8
C : N ratio	10.7 ± 0.3	11.7 ± 0.4	11.0 ± 0.6	11.4 ± 1.3
F_v/F_m	0.72 ± 0.03	0.74 ± 0.04	0.76 ± 0.01	0.76 ± 0.01
$rETR_{max}$	32.8 ± 3.5	31.7 ± 5.0	82.5 ± 13.5	82.4 ± 12.5
I_k (µmol photons m^{-2} s^{-1})	41.2 ± 5.5	38.6 ± 9.6	104.2 ± 20.3	104.4 ± 18.6
α	0.80 ± 0.05	0.84 ± 0.09	0.80 ± 0.03	0.79 ± 0.03

Initial values obtained before acclimation to experiment 2, except length which was obtained after acclimation. F_v/F_m, Optimum quantum yield; $rETR_{max}$, maximum relative electron transport rate; I_k, saturation irradiance; α, photosynthetic efficiency. Mean values ± SD, except mannitol: mean of mean values due to extraction in triplicates, $n = 20$.

Supplementary Table S3 Results of two-way ANCOVA to examine variability of *Laminaria digitata* sporophyte recruitment following gametogenesis.

Parameter	numDF	denDF	*F*-value	*p*-value
Initial density	1	59	72.68	**<0.0001**
Genetic line	9	59	6.92	**<0.0001**
Gametogenesis temp.	1	59	7.38	**0.0086**
Genetic line x gameto temp.	9	59	4.09	**0.0004**

Number of sporophytes per cm² after recruitment was saturated (26 days of gametogenesis treatment) was tested against initial spore density as covariate and interactive effects of genetic line and gametogenesis temperature. numDF, numerator degrees of freedom; denDF, denominator degrees of freedom. Statistically significant values ($p < 0.05$) are indicated in bold text. See also **Supplementary Figure S4**.

5 Publication III

Daniel Liesner, Shivani Rana, Lars Harms, Inka Bartsch, Gernot Glöckner, Klaus Valentin

Evidence for increased heat resilience of intraspecific hybrids compared to inbred lineages of the kelp *Laminaria digitata* (Phaeophyceae) in physiology and transcriptomics

in preparation.

Authors to be included before submission: Gareth Pearson, Sandra Heinrich, Kai Bischof.

Evidence for increased heat resilience of intraspecific hybrids compared to inbred lineages of the kelp *Laminaria digitata* (Phaeophyceae) in physiology and transcriptomics

Daniel Liesner, Shivani Rana, Lars Harms, Inka Bartsch, Gernot Glöckner, Klaus Valentin

ABSTRACT

Marine forests are threatened by ocean warming at the warm distributional edges. Kelps, as the marine forest foundation species, are highly plastic in their thermal responses and may consist of various thermal ecotypes along their latitudinal distribution. To investigate inheritance of thermal traits, we assessed thermal tolerance of inbred (selfings) and outbred (crosses) sporophytes of the kelp *Laminaria digitata* among isolates from the contrasting populations of Helgoland (North Sea) and Spitsbergen (Arctic). First, we investigated the upper thermal tolerance of microscopic sporophytes in a 14-day experiment applying 20–23°C. The upper survival temperature was lower for the inbred Spitsbergen selfing (21°C) than for the Helgoland selfing and the reciprocal crosses (22°C), which indicates mid-parent heterosis in the crosses. We then subjected 4–7 cm long sporophytes to a control temperature (10°C), moderate (19°C) and critical heat stress (20.5°C) to assess metabolic regulation via whole-transcriptome analysis in addition to physiological parameters. Growth and optimum quantum yield decreased in a similar manner in the reciprocal crosses and the Helgoland selfing at 19 and 20.5°C, while inbred Spitsbergen sporophytes died within seven days at these temperatures. Transcriptomic profiles revealed that gene regulation differed among lineages. At 10°C, the Spitsbergen selfing showed the highest gene expression. Considering only the three surviving lineages at 20.5°C, differential gene expression was reduced in the reciprocal crosses compared to the Helgoland selfing, despite their similar physiological responses. Among the identified transcripts, cellular stress responses were reduced in the reciprocal crosses compared to the Helgoland selfing at 20.5°C. These results imply that thermal traits are inherited from both female and male gametophyte parents. In addition, the intraspecific crosses maintained a similar physiology to the inbred Helgoland selfing with reduced metabolic regulation during sublethal heat stress, which may be a beneficial effect of outbreeding. Intraspecific hybrids show potential to provide stable and resilient crops in mariculture. Further, outbreeding may be used in the future to maintain natural populations threatened by ocean warming, but a careful discussion of ethical concerns will become necessary.

INTRODUCTION

Kelps in the brown algal order Laminariales are the foundation of diverse coastal rocky ecosystems known as kelp forests (Teagle et al., 2017). In addition to their immediate ecosystem functions as shelter and food, they sequester significant amounts of carbon (Krause-Jensen and Duarte, 2016), remove anthropogenic nutrients from coastal waters (Kim et al., 2015) and can be of high direct and indirect economic value (Vásquez et al., 2014; Buschmann et al., 2017). These kelp forests are currently threatened by ocean warming especially at their warm range edges (Voerman et al., 2013; Wernberg et al., 2016; Smale et al., 2019), which is a manifestation of the predicted poleward shift of kelp forest distributions under climate change (Raybaud et al., 2013; Assis et al., 2018).

In this study, we investigated the kelp *Laminaria digitata* Hudson J.V. Lamouroux, which grows in the upper sublittoral and infralittoral fringe of cold-temperate and Arctic coasts in the North Atlantic (Lüning, 1990). Like all laminarian kelps, *L. digitata* alternates between microscopic haploid gametophytes and macroscopic diploid sporophytes in a dioecious, anisogamous life cycle. Sex determination occurs in the haploid gametophyte stage (UV sexual system; Coelho et al., 2018) whereas the diploid sporophyte is asexual. Life cycle stages differ in their thermal limits, as gametophytes can tolerate higher temperature (23°C over two weeks; Bolton and Lüning, 1982) than sporophytes (21°C over two weeks; Lüning, 1984; tom Dieck, 1992).

Building on this general knowledge, recent evidence suggests that kelp forest key species are not uniform in their temperature responses, but show signs of adaptation to their local thermal regime (King et al., 2018; Liesner et al., 2020a). Compared to populations from the species' range centre, *L. digitata* populations close to the warm distribution limit show higher heat resilience. The southernmost European population in Quiberon, France, and the population on the North Sea island of Helgoland show higher heat resilience of growth and photoprotective responses, respectively, compared to other populations along the latitudinal distribution of *L. digitata* (Liesner et al., 2020a). This is contrasted by reduced thermal tolerance of the Arctic population of Spitsbergen in photosynthetic quantum yield (Liesner et al., 2020a) and reproductive traits (Martins et al., 2020). This is in accordance with local sea surface temperature (SST) reaching > 18°C in Quiberon (Oppliger et al., 2014) and Helgoland (Bartsch et al., 2013), whereas Kongsfjorden, Spitsbergen reaches maximum SST of 6–7°C (Hanelt et al., 2001; Liesner et al., 2020a). Further, the genetic distance between these populations may have facilitated phenotypic divergence (Liesner et al., 2020a). First evidence on the molecular level shows that heat shock protein expression in South English *L. digitata* populations peaks at higher temperatures than in more northern, Scottish populations (King et al., 2019). Still, it is mostly unclear which mechanisms are driving differences in intraspecific phenotypic responses. Therefore, to improve predictions and mitigation guidelines, temperature responses of kelps and their underlying molecular mechanisms are an important field of study (Heinrich et al., 2015; Li et al., 2019; Monteiro et al., 2019b).

Especially distant and phenotypically diverged populations may provide a useful test system to investigate phenotypic thermal plasticity and inheritance, and the underlying molecular mechanisms.

The underlying process of thermal trait inheritance is still largely unknown in kelps and macroalgae (Martins et al., 2019). Recent research has shown that brown algal gametophytes express different transcriptomic regulatory patterns among sexes generally (Lipinska et al., 2015; Pearson et al., 2019) and in response to temperature (Monteiro et al., 2019a). In the sporophyte stage, there is evidence for beneficial heterosis effects ("hybrid vigour"; Hochholdinger and Hoecker, 2007) in growth and thermal tolerance of hybrid offspring of closely related species (Lüning et al., 1978; tom Dieck and de Oliveira, 1993; Martins et al., 2019). Within species, outbreeding among populations might alleviate effects of inbreeding depression (Raimondi et al., 2004) and may even produce more productive offspring (Westermeier et al., 2010). Heterosis is a known concept to produce stable and viable cultivars in agriculture and mariculture (Li et al., 2007; Westermeier et al., 2010; Fu et al., 2014), but research on the underlying principles of trait inheritance and heterosis is still scarce (Fujimoto et al., 2018). Therefore, producing reciprocal crosses among cultivars may help in understanding mechanisms of thermal trait inheritance in kelps.

In this study, we investigated thermal tolerance of inbred and outbred juvenile *Laminaria digitata* sporophytes among the distant populations of Helgoland and Spitsbergen, which evidently differ in their thermal characteristics (Liesner et al., 2020a; Martins et al., 2020). We hypothesized that inbred lineages (selfings) of Helgoland sporophytes are more tolerant to sublethal high temperature than inbred Spitsbergen sporophytes. Further, we expected outbred hybrids (crosses) among populations to perform intermediate to or better than the inbred lineages (i.e. heterosis). To investigate differentiation and underlying mechanisms of thermal regulation among kelp individuals from different populations in sublethal heat stress, we applied physiological measurements and whole-transcriptome analysis to identify major regulatory processes.

MATERIALS AND METHODS

Experimental design

We designed an experiment using gametophyte isolates from the *L. digitata* populations of Helgoland (North Sea) and Spitsbergen (Arctic), because they represent genetically distant populations thriving at contrasting thermal conditions along the latitudinal gradient (Liesner et al., 2020a). We induced fertilization of gametophytes and performed a first experiment on recruited microscopic sporophytes to define their upper thermal tolerance and differential survival capacity over time of inbred selfings and outbred reciprocal crosses. We then performed a second experiment on 4–7 cm long sporophytes in which we applied control (10°C) and sublethal temperature (19 and 20.5°C) for 18 days. We

measured the physiological traits of growth and photosynthetic optimum quantum yield, and compared transcriptomic responses to critical heat stress at 20.5°C among selfings and crosses.

Culture material, preparation and fertilization

We used unialgal gametophyte cultures isolated in 2015 from spores of one *Laminaria digitata* sporophyte from Kongsfjorden, Spitsbergen, Norway (AWI seaweed culture collection: ♀ 3472, ♂ 3471) and one sporophyte from Helgoland, Germany (AWI seaweed culture collection: ♀ 3436, ♂ 3435). Spitsbergen material had been verified as *L. digitata* morphologically and by DNA barcoding of the sporophyte (ID 78 in Dankworth et al., 2020) to avoid confusion with the morphologically similar Arctic kelp *Hedophyllum nigripes*. Prior to the start of the experiment, cultures were maintained vegetatively under red light (approx. 3 µmol photons m^{-2} s^{-1}; ProfiLux 3 with LED Mitras daylight 150, GHL Advanced Technology, Kaiserslautern, Germany) in a 16:8 h L:D cycle at 15°C in a temperature-controlled cooling chamber (error ± 1°C) in sterile Provasoli-enriched seawater (PES; Provasoli, 1968; modifications: HEPES-buffer instead of TRIS, double concentration of Na_2glycerophosphate; iodine enrichment following Tatewaki, 1966).

To perform fertilization of selfings and crosses, stock suspensions of each gametophyte culture were prepared by gently fragmenting gametophyte material using mortar and pestle. Suspensions were sieved to obtain a fraction of filaments measuring 50–100 µm length. Gametophytes were added to petri dishes (Ø 5 cm) containing four glass cover slips and filled with 12 mL half-strength PES to a desired density of each 250 male and female gametophyte filaments cm^{-2} in all combinations (total 500 gametophytes cm^{-2}). This created the "lineage" treatments of H x H, Helgoland female x Helgoland male; H x S, Helgoland female x Spitsbergen male; S x H, Spitsbergen female x Helgoland male; S x S, Spitsbergen female x Spitsbergen male (n = 4). For all lineages, females are reported first. Additionally, we included female-only samples to control for parthenogenetic development of Helgoland and Spitsbergen females with 500 female gametophyte fragments cm^{-2}. Gametogenesis was induced at 10°C and 15–18 µmol photons m^{-2} s^{-1} white light, which is optimal for both populations (tom Dieck, 1992; Martins et al., 2020). Gametogenesis and sporophyte recruitment were quantified via inverted microscopy (CKX41, Olympus Co., Tokyo, Japan) 14, 21 and 28 days after sowing, when recruitment was saturated. Gametogenesis was quantified based on ontogenetic development of females (vegetative, oogonia, egg release, sporophyte-bearing; Martins et al., 2017). Despite efforts to sow gametophytes at identical densities, Spitsbergen gametophytes (f: 227 ± 22 gametophytes cm^{-2} 14 days after sowing, mean ± SD, n = 8; m: 195 ± 10 gam. cm^{-2}) were about twice as dense as Helgoland gametophytes (f: 120 ± 11 gam. cm^{-2}; m: 95 ± 15 gam. cm^{-2}). Therefore, density was taken into account in the statistical analysis for experiment 1.

Following recruitment, the four cover slips per replicate were divided into four plastic dishes (Coria, Ø 6 cm, polystyrol, VKF Renzel, Germany) to conduct the first experiment on the upper thermal limit of microscopic sporophytes (experiment 1). The remaining sporophytes in the petri dishes were pooled by lineage and were reared for 73 more days first in glass dishes (Ø 9 cm) at the same conditions. Macroscopic sporophytes were subsequently cultivated in 1 L glass beakers and 5 L bottles with gentle aeration at 10°C under increased irradiance of 30–35 µmol photons m^{-2} s^{-1} with weekly changes of half-strength PES medium.

Experiment 1: upper survival temperature of microscopic sporophytes

The four cover slips of one gametogenesis replicate were assigned to one experimental replicate in each temperature treatment of the first experiment to define the upper thermal limit of microscopic sporophytes (20, 21, 22, 23°C). Replicate dishes each containing one coverslip and 100 mL half-strength PES were acclimated at 14°C for 11 days, followed by 18°C for two days before reaching the experimental temperatures in water baths controlled by thermostats (Huber Variostat CC + Pilot ONE, Peter Huber Kältemaschinen GmbH, Offenburg, Germany). Amounts of healthy (fully pigmented), unhealthy (partial bleaching) and dead (fully bleached) sporophytes were counted at the start of the experiment (day 0), after seven and 14 days at the temperature treatments, and after a recovery period of 14 days at 20°C. In each replicate, ~300 sporophytes were randomly counted. Medium was changed at the start of the experiment and at the end of the 14-day temperature treatment. Mid-parent heterosis (MPH; hybrid vigour) of survival in the reciprocal crosses was calculated as the difference between the hybrid response and the average selfing response over both inbred lineages following Hochholdinger and Hoecker (2007):

$$MPH = F_1 - \frac{P_1 + P_2}{2}$$

where F_1 is the trait of a hybrid, and P_1 and P_2 are traits of the parental inbred lineages.

Experiment 2: critical heat stress on macroscopic sporophytes

In the second experiment, 4–7 cm long sporophytes were subjected to temperatures of 10°C (control), 19 and 20.5°C to assess heat stress responses among lineages. These temperatures were chosen based on the results of experiment 1, where the majority of sporophytes survived for 14 days at 20°C and only the Spitsbergen selfing showed reduced resilience at 21°C (**Figure 2**), which corresponds to the published upper thermal limit of *L. digitata* (Bolton and Lüning, 1982; tom Dieck, 1992). Seven sporophytes were assigned to one replicate plastic container (Wide neck containers series 310 PETG, 2000 mL, Kautex GmbH & Co. KG, Bonn-Holzlar, Germany) filled with 1.8 L of half-strength PES (n = 4). Samples were acclimated for one day at 13.5°C and one day at 17°C before reaching the

experimental temperatures of 19 and 20.5°C on day 0 of the experiment, while the control treatments remained at 10°C. Two sporophytes per replicate were marked to be used for growth and fluorometry measurements throughout the experiment by punching small holes in the distal thallus with a Pasteur pipette. Of the unmarked five sporophytes per replicate, three were frozen in liquid nitrogen throughout the experiment (before acclimation, day 1 of temperature treatment, day 18 of temperature treatment). Samples frozen before acclimation and after 18 days of temperature treatment were used for transcriptomic analysis, stored at -80°C and processed within three weeks. The remaining two sporophytes served as backup.

Measurements were conducted on days -2 (before acclimation), 0 (beginning of experiment), 3, 7, 10, 14 and 17. Fluorometric measurements were conducted with an Imaging-PAM (M-Series, MAXI version, Heinz Walz GmbH, Effeltrich, Germany) following 10 minutes dark acclimation. Maximum quantum yield (F_v/F_m) was measured in the meristematic region of two sporophytes per replicate. Fresh weight was quantified after patting dry sporophytes with paper wipes. Relative growth rates were calculated as

$$RGR\ (g\ g^{-1}d^{-1}) = \frac{\ln x_2 - \ln x_1}{t_2 - t_1}$$

where x_1 and x_2 are fresh weights at the successive time points t_1 and t_2, respectively.

Physiological data analysis

For statistical analysis of gametophytic developmental stages, we analysed proportions of ontogenetic stages across lineages and parthenogenesis controls with a chi-square test. We further modelled percentages of gametophytes bearing sporophytes against covariates of female and male gametophyte densities, and lineage as fixed factor. Because the assumption of homogeneity of variances of standardized model residuals was violated, variance structure was included in the models with the weights argument (Zuur et al., 2009) in a generalized least squares model (function "gls") from the R package "nlme" (Pinheiro et al., 2019). To analyse survival of microscopic sporophytes in experiment 1, we calculated fractions of unbleached ("healthy") sporophytes in each replicate to produce survival curves. We then modelled percentages of healthy sporophytes after 14 days of exposure against a density covariate and the fixed factor lineage for each experimental temperature using simple linear models (function "lm"). Relative growth rates and F_v/F_m in experiment 2 were modelled as a function of the fixed factor lineage for each temperature separately at time points 3 (all lineages) and 17 (S x S only included at 10°C because of mortality at 19 and 20.5°C) using simple linear models. All analyses were conducted in R 3.6.0 (R Core Team, 2019).

Sporophyte RNA extraction, sequencing and quality control

For RNA extraction from frozen sporophyte material, we applied the protocol of Heinrich et al. (2012) with modifications described by Heinrich (2018). RNA purity was inspected by NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies LLC, Wilmington, USA) and RNA integrity was confirmed by capillary electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies, Santa Clara, USA). The RNA was enriched for polyA+ species and then converted to a cDNA sequencing library using a TruSeq RNA Library Prep Kit (Illumina, San Diego, USA) according to the manufacturer's protocol. The cDNA was sequenced as 150 bp paired end libraries on an Illumina HiSeq 2500. Reads were quality trimmed with Trimmomatic (Bolger et al., 2014).

Bioinformatic analysis

The *L. digitata* transcriptome was assembled *de novo* from 32 libraries ($n = 3$; $n = 2$ for H x H at 20.5°C) sampled on day 0 and day 18 of experiment 2 using the algorithm ASplice, which returns splicing graphs as a collection of nodes (Sze et al., 2017). Transcript expression was normalized as number of reads per kilobase of node per million reads (RPKM; Sze and Tarone, 2014). Contigs were searched for contamination with bacteria or other eukaryotes using Kraken (Wood and Salzberg, 2014). Contigs ≥ 500 bp were analysed for differential expression using the R package "DESeq2" version 1.28.1 (Love et al., 2014). We used the "ashr" method for shrunken $\log_2$-fold changes ($\log_2$FC) with the requirements of $p < 0.001$ and $\log_2\text{FC} > 2$ to extract differentially regulated genes (DEGs). Comparisons were made only for samples taken on day 18. In total, 16 comparisons were made to investigate different levels of regulation: Temperature effects within the lineages were investigated by comparing each lineage at 20.5°C with their respective 10°C control. Baseline differences in regulation among the lineages were investigated by comparing all lineages at 10°C. Lineage-specific heat responses were investigated by comparing the reciprocal crosses at 20.5°C with the Helgoland selfing at 20.5°C. Due to mortality of the Spitsbergen selfing at 19 and 20.5°C, we further included a cross-comparison of the reciprocal crosses at 20.5°C with the selfings at 10°C to include the Spitsbergen control. All differentially expressed genes were then annotated using the "Trinotate" pipeline (Bryant et al., 2017) and Uniprot Swissprot, Uniref90, RefSeq and NR databases. We used Uniref90 as the primary reference in the functional analysis because it provided the most annotated contigs. When different contigs were annotated as the same gene, potential sequence overlap in the graphical blast output and differing patterns in expression height among samples were used as indicators that contigs belonged to a gene family rather than being fragments of the same gene.

RESULTS

Gametogenesis and recruitment

After 14 days of fertilizing conditions, there were significant differences in the relative proportions of ontogenetic stages among the lineages and the parthenogenesis controls (**Figure 1**; Chi-squared test, $X^2 = 240.31$; df = 15; $p < 0.0001$).

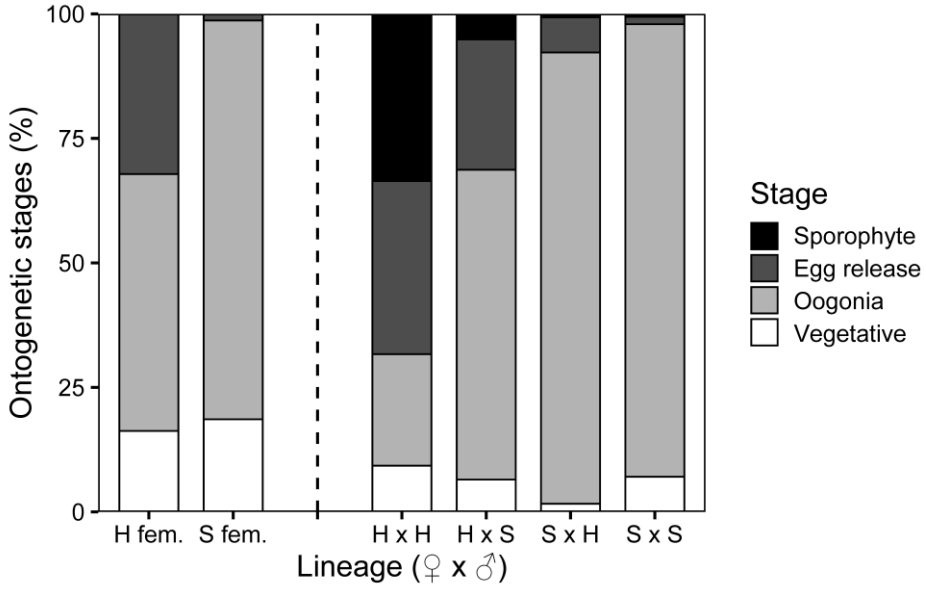


Figure 1 Relative proportions of ontogenetic development of female *Laminaria digitata* gametophytes from Helgoland (H) and Spitsbergen (S) after 14 days at 10°C (mean values, $n = 4$). Parthenogenesis controls (H fem., S fem.) and fertilizing cultures (selfings and crosses) are separated by a dashed line.

Among the four fertilizing lineages (excluding the parthenogenesis controls) there were significant differences in the relative proportion of gametophytes bearing sporophytes ($p < 0.0001$, **Table 1**). The Helgoland selfings contained a mean of 34% sporophyte-stage gametophytes, compared to 5% in the H x S cross, 0.7% in S x H and 0.6% in the Spitsbergen selfing (Tukey tests, H x H > (H x S = S x S = S x H); $p < 0.01$). Gametophytes which had developed to at least the stage of egg release made up 68% of the Helgoland selfing, 31% of H x S, 8% of S x H and 2% of the Spitsbergen selfing. 2–9% of gametophytes remained vegetative across lineages. The significant covariate for female gametophyte density ($p = 0.0001$, **Table 1**) described a higher proportion of gametophytes bearing sporophytes at lower female densities. The faster development of Helgoland females was also visible in the parthenogenesis controls. After 28 days, recruitment was saturated as means of 75–80% (S x S, S x H, H x S) and 90% (H x H) of females had developed to the sporophyte stage (ANCOVA, $F(3,11) = 8.13$, $p = 0.0039$).

Table 1 Results of generalized least squares model to examine ontogenetic development of *Laminaria digitata* among lineages.

Fixed effect	DFn	DFd	*F*-value	*p*-value
Female density (covariate)	1	10	43.44	**0.0001**
Male density (covariate)	1	10	2.50	0.1448
Lineage	3	10	43.24	**<0.0001**

Percentage of gametophytes bearing sporophytes after 14 days of fertilizing culture was tested against covariates of female and male gametophyte densities, and against lineage as fixed factor. DFn and DFd denote numerator and denominator degrees of freedom, respectively. Statistically significant values ($p < 0.05$) are indicated in bold.

Upper survival temperature of microscopic sporophytes (experiment 1)

In **Figure 2**, we show the percentage of fully pigmented ("healthy") microscopic sporophytes for each lineage over 14 days of heat treatment and subsequently 14 days of recovery at 20°C in four temperature treatment panels. While almost all sporophytes survived at 20°C for 14 days, 23°C was lethal for most sporophytes across lineages. At 20°C, lineage did not have a significant effect as 99–100% of sporophytes were healthy in all lineages after 14 days ($p = 0.4164$, **Table 2**), but in the Spitsbergen selfing the fraction of healthy sporophytes decreased to 83% during the subsequent 14 days of recovery at 20°C. Major temperature effects were visible in S x S at 21°C, which significantly decreased the fraction of healthy sporophytes to 68% after 14 days compared to all other lineages, which remained 93–99% healthy ($p < 0.0001$, **Table 2**; Tukey tests, $p < 0.0001$). Again, health of S x S sporophytes further decreased to 28% during recovery. At 22°C, health of S x S sporophytes decreased drastically to 6%, while the other crosses remained at 70–80%, and the Helgoland selfing retained 90% healthy sporophytes ($p < 0.0001$, **Table 2**; Tukey tests, H x H > (S x H = H x S) > S x S, $p < 0.05$). At 23°C, percentages of healthy sporophytes among lineages ranged between 0% and 3% and were not significantly different ($p = 0.0600$, **Table 2**). Only in the Helgoland selfing, 4.5% of healthy sporophytes persisted during recovery. These results led to the decision to perform experiment 2 with a control temperature of 10°C, 19°C as a moderate heat treatment and 20.5°C as a critical but sublethal heat treatment.

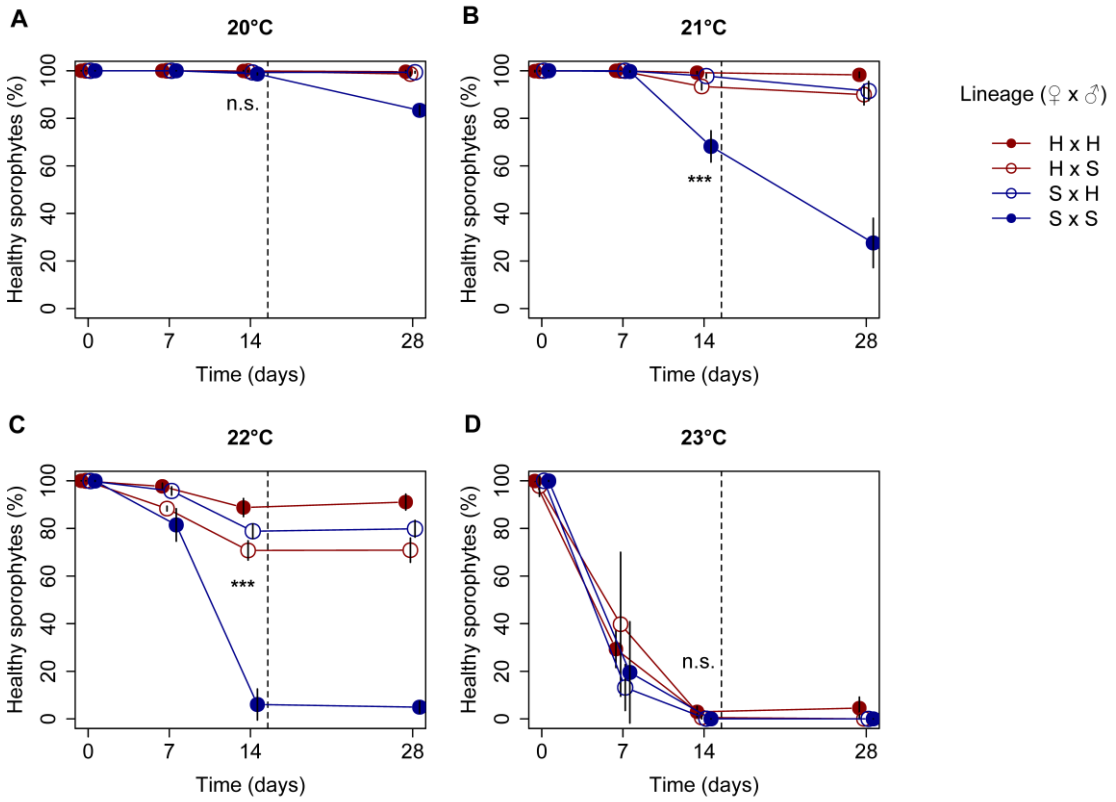


Figure 2 Relative proportion of fully pigmented (healthy) *Laminaria digitata* sporophytes of different lineages in experiment 1 over 14 days in treatments of 20°C (**A**), 21°C (**B**), 22°C (**C**) and 23°C (**D**) followed by 14 days of recovery at 20°C (separated by dashed line; mean values ± SD, $n = 4$). Significance of the fixed effect lineage on sporophyte health after 14 days of temperature exposure is indicated in the Figure; ***, $p < 0.001$; n.s., not significant. For pairwise comparisons, see main text.

The comparably high survival of the reciprocal crosses at 21 and 22°C can be quantified as mid-parent heterosis (MPH), in which fitness of hybrid offspring is significantly better than the average value of the two parental inbred lines (Hochholdinger and Hoecker, 2007). Over 14 days at 21°C, H x S showed MPH of 10% ± 3% (mean ± SD) higher survival than the average selfing response over both populations, and a 23% ± 3% higher survival at 22°C. S x H showed MPH of 14% ± 6% higher survival than the average selfing response at 21°C and a 31% ± 6% higher survival at 22°C.

Table 2 Results of linear models to examine fractions of unbleached *Laminaria digitata* sporophytes in the upper survival temperature experiment on microscopic sporophytes (experiment 1).

Temp. (°C)	Fixed effect	DFn	DFd	*F*-value	*p*-value
20	Sporophyte density (covariate)	1	11	8.41	**0.0144**
	Lineage	3	11	1.04	0.4146
21	Sporophyte density (covariate)	1	11	119.45	**<0.0001**
	Lineage	3	11	32.44	**<0.0001**
22	Sporophyte density (covariate)	1	11	632.90	**<0.0001**
	Lineage	3	11	111.75	**<0.0001**
23	Sporophyte density (covariate)	1	11	5.06	**0.0460**
	Lineage	3	11	3.33	0.0600

Percentages of healthy sporophytes per sample after 14 days of exposure were tested against a density covariate and against the fixed factor lineage in separate models for each temperature treatment. DFn and DFd denote numerator and denominator degrees of freedom, respectively. Statistically significant values ($p < 0.05$) are indicated in bold.

Heat stress experiment on macroscopic sporophytes (experiment 2)

In experiment 2, we aimed to identify differences among lineages in response to each temperature treatment. Due to the unbalanced design following the loss of the Spitsbergen selfing at 19 and 20.5°C, we analysed differences among lineages within each temperature treatment on day 3 (including the Spitsbergen selfing), and on day 17 (excluding the Spitsbergen selfing at 19 and 20.5°C). Relative growth rates of fresh weight (RGR) decreased over time for all lineages at 19 and 20.5°C (**Figure 3**). We show RGR over the acclimation period (days -2–0) and over 17 days of heat treatment. Each point refers to growth rates over the time period starting from the previous sampling date. Over the first three days at 10°C, growth rates among lineages were not significantly different ($p = 0.9521$, **Table 3**) and all ranged > 0.12 g g^{-1} d^{-1}. At 19°C, growth of the Spitsbergen selfing was significantly reduced to 0.06 g g^{-1} d^{-1} compared to all other lineages at 19°C, which ranged around 0.1 g g^{-1} d^{-1} ($p < 0.0001$, **Table 3**; Tukey tests, $p < 0.001$). At 20.5°C, growth of the Spitsbergen selfing was again significantly reduced at < 0.05 g g^{-1} d^{-1} compared to all other lineages ($p = 0.0008$, **Table 3**; Tukey tests, $p < 0.01$), which retained similar growth around 0.07 g g^{-1} d^{-1}. Following day 3, all sporophytes of the Spitsbergen selfing bleached at 19 and 20.5°C and were therefore removed from the analysis. Between days 14 and 17, growth rates differed significantly among lineages at 10°C ($p = 0.0154$, **Table 3**). H x S sporophytes grew significantly faster at 0.09 g g^{-1} d^{-1} than S x S at 0.07 g g^{-1} d^{-1} (Tukey tests, (H x S = S x H = H x H) > (S x H = H x H = S x S), $p < 0.01$). At 19°C, growth rates among the three remaining lineages did not differ significantly ($p = 0.3885$, **Table 3**). At 20.5°C, growth rates differed significantly among lineages (p = 0.0296, **Table 3**), in that H x S retained slightly higher growth at 0.02 g g^{-1} d^{-1} compared to the Helgoland selfing at 0.01 g g^{-1} d^{-1} (Tukey tests, (H x S = S x H) > (S x H = H x H), p < 0.05).

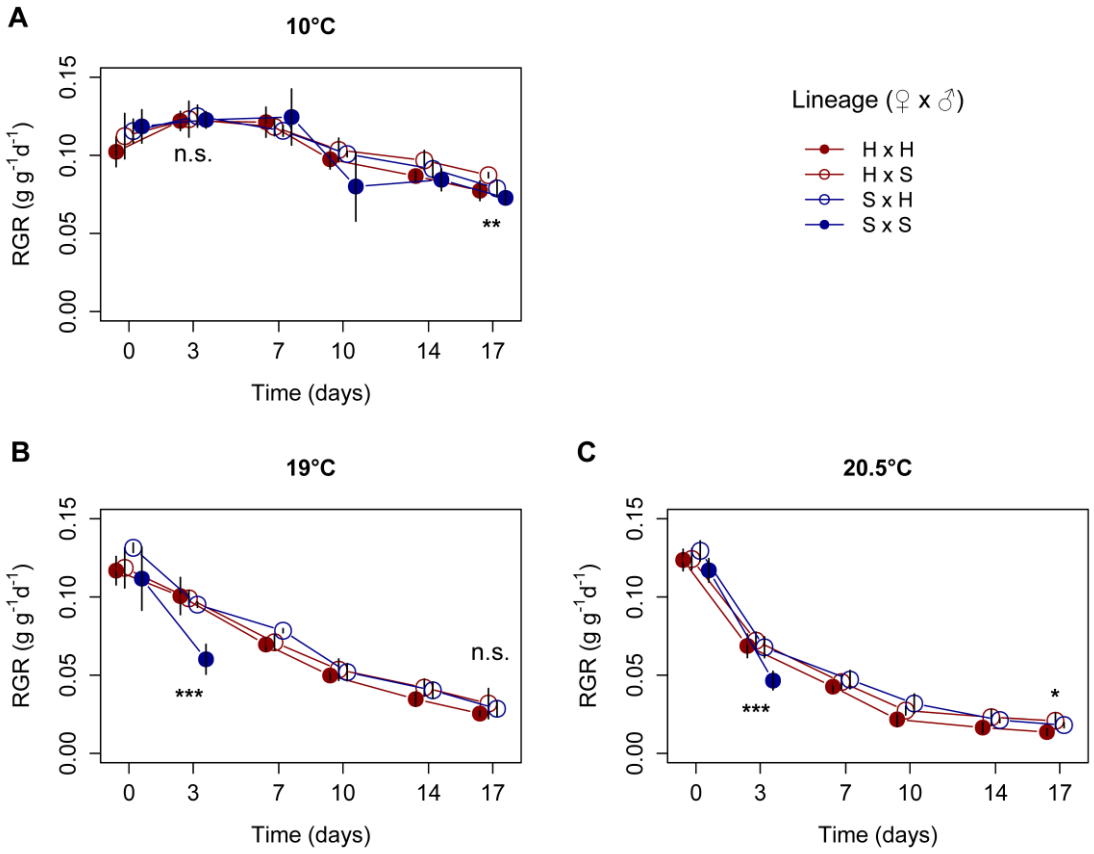


Figure 3 Relative growth rates of *Laminaria digitata* sporophytes from different lineages over time at 10°C (**A**), 19°C (**B**) and 20.5°C (**C**) in experiment 2 (mean ± SD, $n = 4$). Significance of the fixed effect lineage on sporophyte growth after 3 and 17 days of temperature exposure is indicated in the Figure; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s., not significant. For pairwise comparisons, see main text.

Table 3 Results of linear models to examine relative growth rates among lineages for two time points in the critical heat stress experiment on macroscopic *Laminaria digitata* sporophytes (experiment 2).

Temp.	Fixed	Day 3				Day 17			
(°C)	effect	DFn	DFd	*F*-value	*p*-value	DFn	DFd	*F*-value	*p*-value
10	Lineage	3	12	0.11	0.9521	3	12	6.42	**0.0154**
19	Lineage	3	12	22.59	**<0.0001**	2	9	1.05	0.3885
20.5	Lineage	3	12	13.12	**0.0008**	2	9	5.34	**0.0296**

Relative growth rates of fresh weight between day 0 and day 3, and between day 14 and day 17 were tested against the fixed factor lineage in separate models for each temperature treatment. DFn and DFd denote numerator and denominator degrees of freedom, respectively. *p*-values were adjusted for multiple comparisons with FDR corrections. Statistically significant values ($p < 0.05$) are indicated in bold.

Optimum quantum yield F_v/F_m was relatively stable across lineages and temperatures and was mostly in a healthy range of 0.6–0.7 (**Figure 4**). On day 3, F_v/F_m differed significantly among lineages at all temperatures. At 10°C, F_v/F_m was significantly lower in the Spitsbergen selfing at 0.64 than in the other lineages, which ranged from 0.66–0.67 ($p = 0.0006$, **Table 4**; Tukey tests, $p \leq 0.01$). At 19°C, F_v/F_m was significantly highest in the Helgoland selfing at 0.70 and lowest in the Spitsbergen selfing at 0.67, while the reciprocal crosses were intermediate at 0.68 ($p = 0.0004$, **Table 4**; Tukey tests, H x H > (S x H = H x S) > (H x S = S x S), $p < 0.05$). At 20.5°C, F_v/F_m was significantly lower in the Spitsbergen selfing at 0.65 than in the Helgoland selfing for which F_v/F_m was 0.70 ($p = 0.0414$, **Table 4**; Tukey tests, (H x H = S x H = H x S) > (S x H = H x S = S x S), $p < 0.05$). On day 17, F_v/F_m did not differ significantly among lineages at 10°C ($p = 0.0629$, **Table 4**) and ranged between 0.67–0.69. At 19°C, F_v/F_m differed significantly among lineages ($p = 0.0218$, **Table 4**), in that F_v/F_m was higher in S x H at 0.72 than in H x S and the Helgoland selfing at 0.70 (Tukey tests, S x H > (H x S = H x H), $p < 0.05$). At 20.5°C, F_v/F_m ranged between 0.68–0.71 and again did not differ significantly among lineages ($p = 0.4549$, **Table 4**).

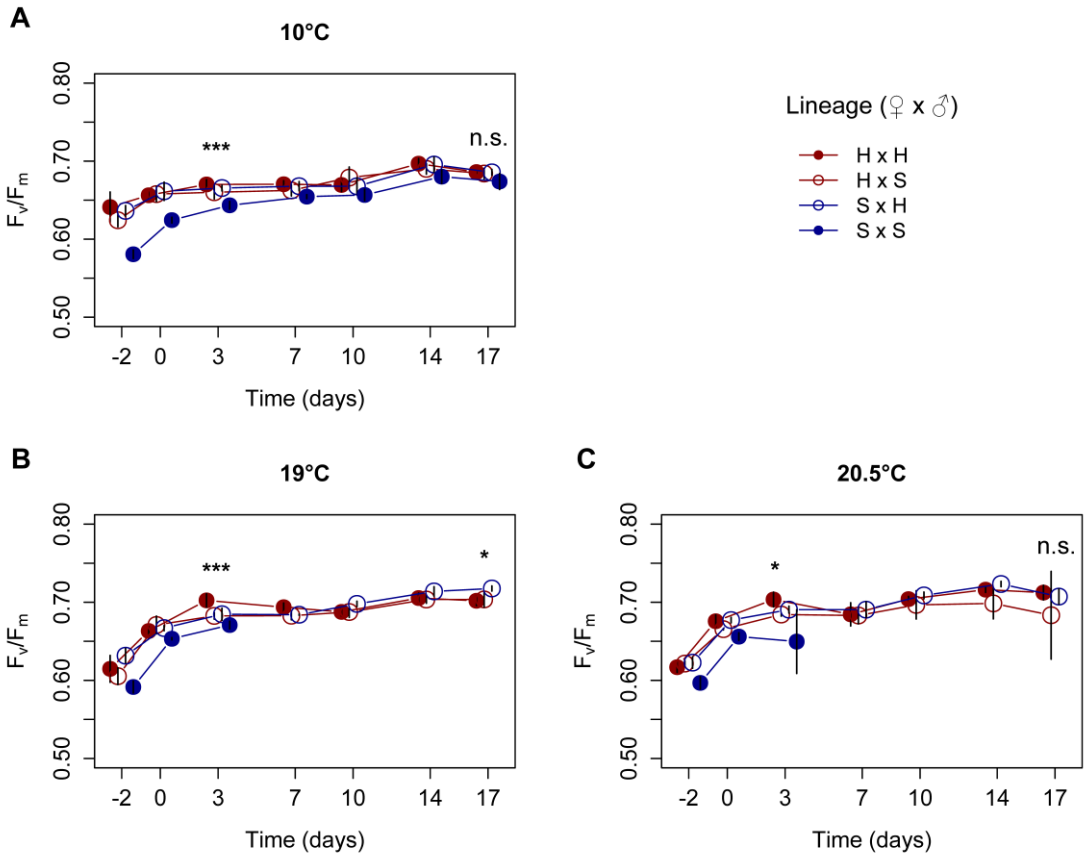


Figure 4 Optimum quantum yield F_v/F_m of *Laminaria digitata* sporophytes from different lineages over time at 10°C (**A**), 19°C (**B**) and 20.5°C (**C**) in experiment 2 (mean ± SD, $n = 4$). Significance of the fixed effect lineage on F_v/F_m after 3 and 17 days of temperature exposure is indicated in the Figure; ***, $p < 0.001$; *, $p < 0.05$; n.s., not significant. For pairwise comparisons, see main text.

Table 4 Results of generalized least squares model to examine optimum quantum yield (F_v/F_m) among lineages for two time points in the critical heat stress experiment on macroscopic *Laminaria digitata* sporophytes (experiment 2).

Temp.	Fixed	Day 3				Day 17			
(°C)	effect	DFn	DFd	*F*-value	*p*-value	DFn	DFd	*F*-value	*p*-value
10	Lineage	3	12	14.72	**0.0006**	3	12	3.19	0.0629
19	Lineage	3	12	15.81	**0.0004**	2	9	6.03	**0.0218**
20.5	Lineage	3	12	4.76	**0.0414**	2	9	0.86	0.4549

F_v/F_m on day 3 and day 17 was tested against the fixed factor lineage in separate models for each temperature treatment. DFn and DFd denote numerator and denominator degrees of freedom, respectively. *p*-values were adjusted for multiple comparisons with FDR corrections. Statistically significant values ($p < 0.05$) are indicated in bold.

Transcriptome Quality

The number of reads per library ranged between 28 million and 44 million with an average of 33.5 million reads per library. The raw assembled transcriptome contained 598,395 contigs (NODEs). Following a cutoff at ≥ 500 bp, 23,288 contigs remained and were used for the differential expression analyses. Principal component analysis revealed that all replicates of the same lineage and temperature treatment clustered well (**Figure 5**). Distribution of data along the x-axis (PC1) explained 40% of the variance among gene expression in the samples, and mainly separated the two temperature treatments. Distribution along the y-axis (PC2) explained 27% of the variance and divided samples based on origin of the parental gametophytes. While the Helgoland and Spitsbergen selfings each formed clearly separated clusters within the temperature treatments, the reciprocal crosses grouped together at 10°C, but were slightly separated at 20.5°C. The placement of the reciprocal crosses between both selfings verifies that outbreeding was successful for the tested sporophytes.

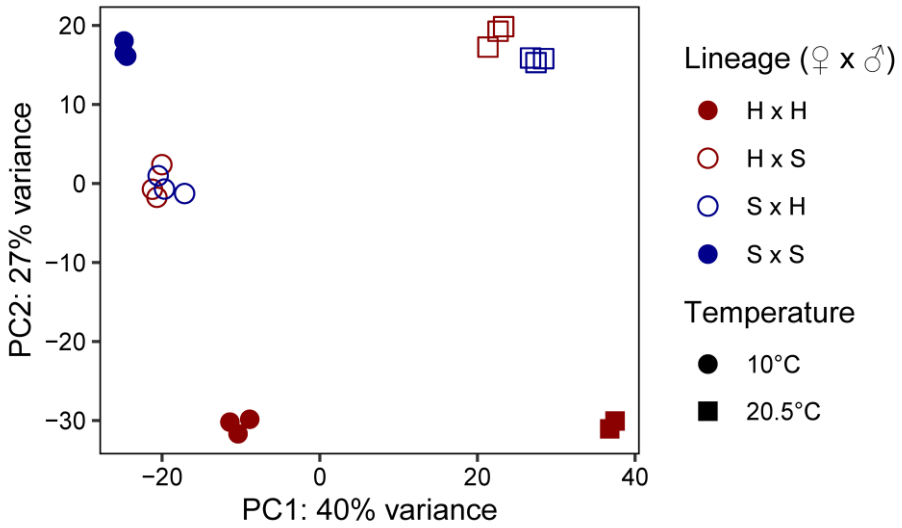


Figure 5 Principal component analysis of normalized read counts in all combinations of lineage and temperature treatment of *Laminaria digitata* sporophytes (n = 3; for H x H at 20.5°C, n = 2).

Differential gene expression in experiment 2

Out of the 23,288 contigs, we extracted 871 (3.74%) differentially expressed contigs (differentially expressed genes, DEGs) among all comparisons in the differential expression analysis. The number of differentially expressed genes differed among comparisons and a pattern of reduced gene regulation in the reciprocal crosses became evident. We assessed general heat responses by analysing differential gene expression between 20.5 and 10°C within each lineage (**Figure 6A**). Gene regulation in response to 20.5°C was lowest in H x S, where 76 genes were up-regulated and 120 genes were down-regulated compared to the 10°C control. In S x H at 20.5°C, 152 genes were up-regulated and 83 genes were down-regulated, which is in a comparable range to the 162 up-regulated and 86 down-regulated genes in the Helgoland selfing at 20.5°C. To identify general patterns among cultivars of the different locations, we conducted comparisons of the Spitsbergen selfing and of both reciprocal crosses against the Helgoland selfing at 10°C, and comparisons of both crosses against the Spitsbergen selfing at 10°C (**Figure 6B**). In the Spitsbergen selfing, regulation was strongest at 10°C as 138 genes were up-regulated and 53 genes were down-regulated compared to the Helgoland selfing. In contrast, both reciprocal crosses showed reduced gene regulation compared to either selfing. H x S up-regulated 35 genes and down-regulated 29 genes at 10°C, whereas S x H up-regulated 37 genes and down-regulated 10 genes at 10°C compared to the Helgoland selfing at 10°C. When compared to the Spitsbergen selfing at 10°C as control, H x S up-regulated 15 genes and down-regulated 34 genes, whereas S x H up-regulated 18 genes and down-regulated 20 genes.

To investigate potential population-specific regulatory heat responses among the reciprocal crosses, we first compared both crosses to the Helgoland selfing at 20.5°C (**Figure 6C**). Compared to H x H at 20.5°C, both crosses up-regulated fewer genes than were down-regulated at 20.5°C. In H x S, 36 genes were up-regulated while 161 genes were down-regulated. Similarly, in S x H, 38 genes were up-regulated while 98 genes were down-regulated. We then compared both reciprocal crosses to both Helgoland and Spitsbergen selfings at 10°C in a cross-comparison of lineage and temperature effects (**Figure 6C**) to compensate for the loss of the Spitsbergen selfing control at 20.5°C. Compared to H x H at 10°C, S x H at 20.5°C showed the highest regulation with 267 up-regulated and 119 down-regulated genes, whereas in H x S at 20.5°C, 134 genes were up-regulated and 162 genes were down-regulated. Against the S x S control at 10°C, H x S up-regulated 81 genes and down-regulated 239 genes at 20.5°C, whereas S x H up-regulated 94 genes and down-regulated 116 genes.

Finally, we performed direct comparisons between the reciprocal crosses at 10°C and at 20.5°C (data not shown), which revealed minimal differential regulation among the crosses. In a direct comparison of H x S at 10°C against S x H at 10°C, only one gene was significantly up-regulated, while another gene was significantly down-regulated. In a direct comparison of H x S at 20.5°C against S x H at 20.5°C, only two genes were significantly up-regulated and nine genes were significantly down-regulated. Of the DEGs reported in these comparisons, none were functionally annotated.

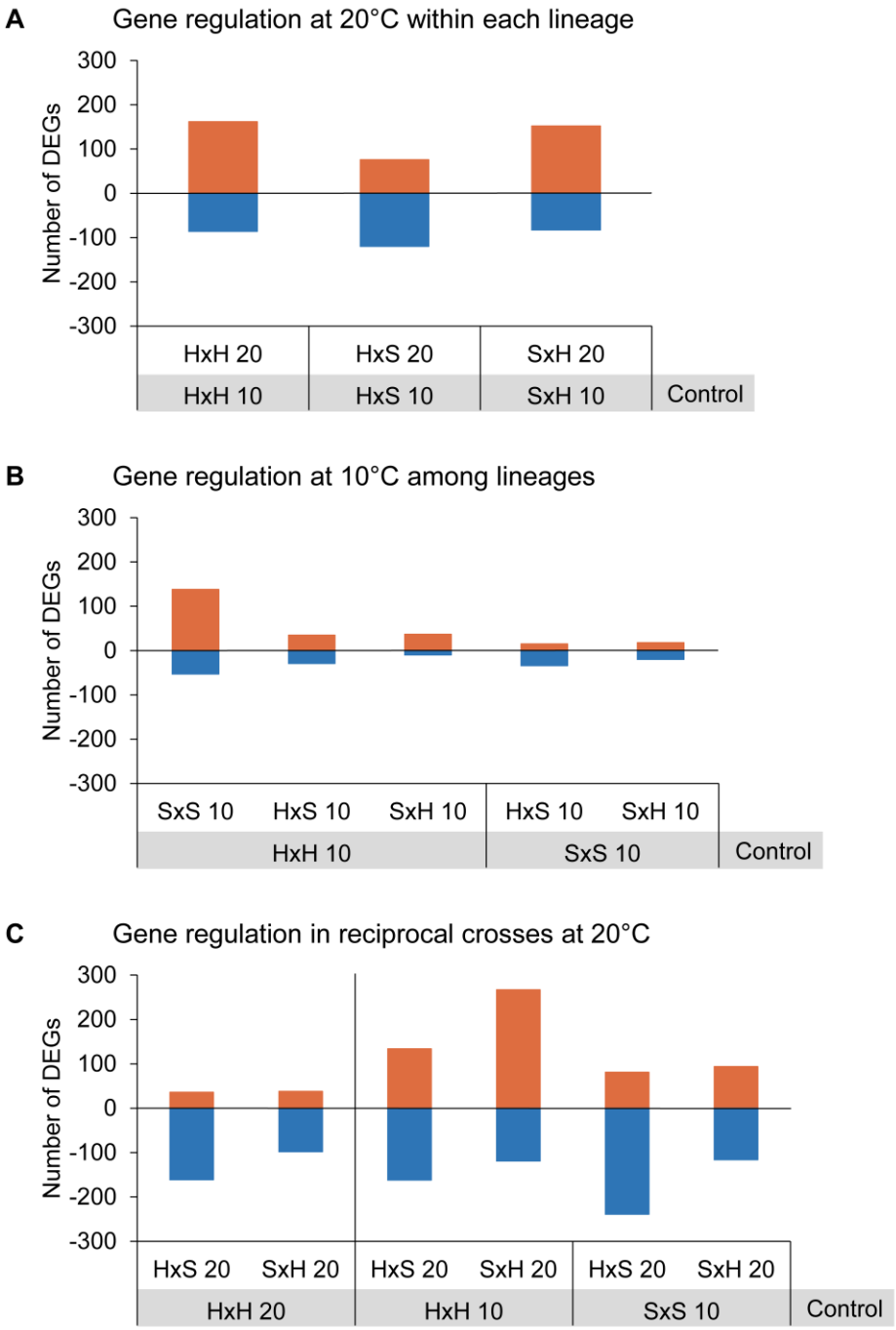


Figure 6 Number of significantly up-regulated (orange) and down-regulated (blue) differentially expressed genes (DEGs; $p < 0.001$, $\log_2 FC \geq 2$) in *Laminaria digitata* sporophytes among comparisons of treatments (x-axis, upper row) and controls (x-axis, lower row) in experiment 2. Treatment abbreviations are composed as female x male from Helgoland (H) and Spitsbergen (S) at 10°C (10) and 20.5°C (20). (**A**) Comparisons within lineages across temperature treatments of 20.5 and 10°C to visualize temperature effects within lineages. (**B**) Comparisons of the Spitsbergen selfing and both reciprocal crosses at 10°C against the Helgoland selfing at 10°C, and of both reciprocal crosses against the Spitsbergen selfing at 10°C to visualize baseline differences in regulation. (**C**) Comparisons of the reciprocal crosses at 20.5°C against the Helgoland selfing at 20.5°C, and against both Helgoland and Spitsbergen selfings at 10°C as controls (cross-comparisons, separated by a vertical line) to visualize lineage-specific heat responses. Two comparisons were omitted for clarity due to low regulation (HxS 10 vs. SxH 10; HxS 20 vs. SxH 20).

Functional analysis of differentially expressed genes

Among the 871 differentially regulated genes, 355 were annotated with the UniRef90 database, of which 154 transcripts were assigned a putative gene product (collected in **Supplementary Table 1**). Due to the low number of annotated DEGs, we manually classified and examined the DEGs in the different comparisons and focused our analysis on key genes and main metabolic processes. To investigate regulatory responses among within-population selfings and reciprocal crosses, we compiled annotated DEGs into Tables distinguished by the major functional groups of lipid metabolism (**Table 5**), carbon assimilation, carbohydrate and cell wall metabolism (**Table 6**), photosynthesis and pigment metabolism (**Table 7**), and cellular stress responses (**Table 8**).

Lipid metabolism was regulated by all lineages in response to heat (**Table 5**). All lineages responded to the 20.5°C treatment by up-regulating lipase genes compared to their 10°C controls. One lipase gene was additionally up-regulated in all lineages involving Spitsbergen at 10°C compared to the Helgoland selfing at 10°C. At 20.5°C, expression of lipase was reduced in the reciprocal crosses compared to the Helgoland selfing. Genes for Acyl-CoA synthetases, which are involved in lipid catabolism, were up-regulated in the Spitsbergen selfing at 10°C and in the S x H cross at 20.5°C compared to the Helgoland selfing at 10 and 20.5°C. A decrease in polyunsaturated fatty acids (PUFAs) in response to heat is indicated by the down-regulation of a fatty acid desaturase gene within all lineages at 20.5°C compared to 10°C. Further, two lipoxygenase genes were down-regulated in response to 20.5°C in H x H and in H x S compared to both selfings at 10°C, as well as in both reciprocal crosses compared to the Helgoland selfing at 20.5°C.

Genes associated cell wall metabolism were down-regulated at 20.5°C (**Table 6**). Within each lineage, three to four genes for mannuronan C-5 epimerase were down-regulated at 20.5°C compared to 10°C. Further, two cellulose synthase genes were down-regulated only in the Helgoland selfing at 20.5°C compared to 10°C. A glycosyltransferase gene was down-regulated in all comparisons of 20.5°C treatments against 10°C controls. Related to the assimilation of inorganic carbon, a gene coding for alpha-carbonic anhydrase was up-regulated in all lineages involving Spitsbergen at 10°C and in the S x H cross at 20.5°C compared to the Helgoland selfing at 10°C. In general carbohydrate metabolism, two genes for endo-1,3-beta-glucanase were up-regulated in the H x S cross at 20.5°C compared to the Helgoland selfing at 20.5°C, and in both reciprocal crosses at 20.5°C compared to the Helgoland selfing at 10°C, which indicates an increased degradation of laminarin. Further, the expression of pyruvate kinase, a key enzyme of glycolysis, was up-regulated in both reciprocal crosses at 20.5°C compared to the Spitsbergen selfing at 10°C.

Expression of genes related to chlorophyll synthesis was reduced in response to heat (**Table 7**). A magnesium chelatase subunit H gene was significantly down-regulated at 20.5°C compared to 10°C within all lineages. Further, a protochlorophyllide reductase gene was down-regulated in response to

20.5°C across all lineages involving Helgoland in comparison to various 10°C controls. In comparison, genes involved in production of thylakoid membranes were up-regulated in response to 20.5°C, as shown by the up-regulation of a monogalactosyldiacylglycerol synthase gene in the reciprocal crosses at 20.5°C. Further, the expression of a thylakoid lumenal 15 kDa protein was up-regulated in the S x H cross at 20.5°C compared to the Helgoland selfing at 10°C. A gene coding for a light harvesting complex (LHC) protein was up-regulated in both reciprocal crosses at 20.5°C compared to the Helgoland selfing at 10°C, while a gene for photosystem I reaction center subunit XI was down-regulated in the crosses at 20.5°C compared to the Spitsbergen selfing at 10°C. A gene coding for a violaxanthin-de-epoxidase (VDE) domain-containing protein was down-regulated in the Helgoland selfing and the H x S cross in response to 20.5°C. The expression of a Rieske (2Fe-2S) region protein was significantly up-regulated in all lineages involving Spitsbergen compared to the Helgoland selfing at 10 and 20.5°C.

Genes related to cellular stress responses did not show clear regulation patterns among lineages and temperatures (**Table 8**). For instance, a gene coding for heat shock protein 70 was only significantly up-regulated in the H x S cross at 20.5°C compared to the Spitsbergen selfing at 10°C. A putative dehydroascorbate reductase gene was up-regulated in the S x H cross at 20.5°C compared to the Helgoland selfing and S x H at 10°C. The expression of superoxide dismutase was down-regulated in both reciprocal crosses at 20.5°C compared to the Helgoland selfing at 20.5°C. Further, superoxide dismutase was down-regulated in both crosses at 10°C compared to the Spitsbergen selfing at 10°C and was only up-regulated within the Helgoland selfing at 20.5°C compared to 10°C. A gene coding for alternative oxidase isoform A was up-regulated within the Helgoland selfing at 20.5°C compared to 10°C, but was down-regulated in the H x S cross at 20.5°C compared to the Helgoland selfing at 20.5°C. A glutaredoxin gene was down-regulated in the reciprocal crosses at 20.5°C compared to the Spitsbergen selfing at 10°C, while a peroxidase gene was down-regulated in the Helgoland selfing at 20.5°C compared to 10°C. Three genes coding for putative respiratory burst oxidase homolog proteins were down-regulated in both reciprocal crosses at 20.5°C compared to the Helgoland selfing at 10 and 20.5°C.

Table 5 Log_2-fold changes for differentially expressed genes with annotated function in regulating lipid metabolism across lineages and temperature treatments of *Laminaria digitata* sporophytes in experiment 2.

		20°C within lineages			10°C among lineages					20°C crosses		20°C crosses vs. 10°C controls			
	sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
Putative gene product	**control →**	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Acyl-CoA synthetase *		.	.	.	.	.	.	.	.	.	.	.	2.1	.	.
Acyl-CoA synthetase *		.	.	.	.	.	.	.	.	.	.	.	2.0	.	.
Acyl-CoA synthetase *		.	.	.	2.0	.	.	.	.	.	.	.	2.2	.	.
Elongation of fatty acids protein		.	.	.	.	.	.	.	.	.	.	.	.	-2.2	-2.1
Fatty acid desaturase		-2.2	-2.0	-2.1	.	.	.	.	.	.	.	.	-2.1	.	.
Lipase *		2.3	3.0	3.5	.	.	.	.	.	.	.	.	.	5.4	5.3
Lipase *		4.5	.	.	.	.	.	.	.	-5.0	-3.5	.	.	.	.
Lipase		2.7	.	.	4.5	3.3	3.8	.	.	.	.	4.2	3.8	.	.
Lipoxygenase		-3.4	.	.	.	.	.	.	.	-5.4	-3.8	-3.4	.	-3.1	.
Lipoxygenase		-3.4	.	.	.	.	.	.	.	-5.2	-3.6	-3.4	.	-2.4	.
Long-chain acyl-CoA synthetase		.	.	2.0	.	.	.	.	.	.	4.6	.	6.0	.	.

Numbers indicate log_2-fold changes and colours indicate significant up- (orange) or down-regulation (blue) of genes ($log_2FC \geq 2$; $p < 0.001$). Treatment abbreviations are composed as female x male from Helgoland (H) and Spitsbergen (S) at 10°C (10) and 20.5°C (20). Two comparisons were omitted for clarity due to low regulation (HxS 10 vs. SxH 10; HxS 20 vs. SxH 20). * denotes contigs which were annotated with the same Uniref ID and could not be confirmed as separate genes.

Table 6 Log_2-fold changes for differentially expressed genes with annotated function in regulating carbon assimilation, carbohydrate and cell wall metabolism across lineages and temperature treatments of *Laminaria digitata* sporophytes in experiment 2.

		20°C within lineages			10°C among lineages					20°C crosses		20°C crosses vs. 10°C controls			
	sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
Putative gene product	**control →**	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Carbonic anhydrase		.	.	.	3.4	2.4	2.6	.	.	.	.	.	2.0	.	.
Cellulose synthase (UDP-forming), family GT2		-2.5	.	.	.	.	.	.	.	.	.	.	.	.	.
Cellulose synthase (UDP-forming), family GT2		-2.3	.	.	.	.	.	.	.	.	.	.	.	.	.
Endo-1,3-beta-glucanase, family GH81		.	4.1	.	.	.	.	.	.	6.0	.	6.3	4.8	.	.
Endo-1,3-beta-glucanase, family GH81		.	3.5	.	.	.	.	.	.	5.7	.	6.1	4.6	.	.
Glycosyltransferase, family GT4		-2.8	-3.1	-2.4	.	.	.	.	.	.	.	-2.7	-2.7	-2.5	-2.5
Mannuronan C-5 epimerase		-3.7	-3.7	-4.3	.	.	.	.	.	.	.	-2.7	-3.2	-3.1	-3.7
Mannuronan C-5 epimerase		-2.7	.	.	.	.	.	.	.	.	.	.	.	-2.1	-2.1
Mannuronan C-5 epimerase		-3.5	-2.4	-2.6	.	.	.	.	.	.	.	-2.3	-2.9	.	-2.4
Mannuronan C-5 epimerase N-terminal		.	-4.2	-3.3	.	.	.	.	.	.	.	-5.1	-4.7	-4.5	-4.0
Pyruvate kinase		.	.	.	.	.	.	.	.	.	.	.	.	2.5	2.6
Unsaturated glucuronyl hydrolase, family GH88		.	.	.	.	.	.	.	.	-3.1	.	-2.5	.	-2.3	.

Numbers indicate log_2-fold changes and colours indicate significant up- (orange) or down-regulation (blue) of genes ($log_2FC \geq 2$; $p < 0.001$). Treatment abbreviations are composed as female x male from Helgoland (H) and Spitsbergen (S) at 10°C (10) and 20.5°C (20). Two comparisons were omitted for clarity due to low regulation (HxS 10 vs. SxH 10; HxS 20 vs. SxH 20). * denotes contigs which were annotated with the same Uniref ID and could not be confirmed as separate genes.

Table 7 Log_2-fold changes for differentially expressed genes with annotated function in regulating photosynthesis and pigment metabolism across lineages and temperature treatments of *Laminaria digitata* sporophytes in experiment 2.

	20°C within lineages			**10°C among lineages**					**20°C crosses**		**20°C crosses vs. 10°C controls**			
sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
control →	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Putative gene product														
High-chlorophyll fluorescence 101 HCF101; ATP binding	.	.	2.3	.	.	.	.	.	.	.	.	2.1	.	.
Light harvesting complex protein	.	.	.	.	.	.	.	.	.	.	2.0	2.2	.	.
Magnesium chelatase subunit H, putative chloroplast *	-2.8	-2.3	-2.2	.	.	.	.	.	.	.	.	-2.2	.	.
Magnesium chelatase subunit H, putative chloroplast *	-3.3	-2.1	-2.3	.	.	.	.	.	.	.	.	-2.6	.	-2.1
Monogalactosyldiacylglycerol synthase, family GT28	.	3.7	3.8	.	.	.	.	.	4.8	5.1	5.3	5.9	2.5	3.1
Photosystem I reaction center subunit XI	.	.	.	.	.	.	.	.	.	.	.	.	-4.2	-3.6
Protochlorophyllide reductase, putative chloroplast *	-2.8	-2.5	.	.	.	.	.	.	.	.	-2.3	.	-2.3	.
Protochlorophyllide reductase, putative chloroplast *	-3.0	.	.	.	.	.	.	.	.	.	.	-2.0	.	.
Rieske (2Fe-2S) region	.	.	.	6.8	6.0	5.7	.	.	6.4	4.9	5.7	4.8	.	.
Thylakoid lumenal 15 kDa protein, chloroplast (P15)	.	.	.	.	.	.	.	.	.	.	.	2.1	.	.
VDE domain-containing protein *	-2.2	-2.0	.	.	.	.	.	.	.	.	-2.1	.	.	.
VDE domain-containing protein *	-2.4	-2.2	.	.	.	.	.	.	.	.	-2.4	.	-2.3	.
VDE domain-containing protein *	-2.4	-2.4	.	.	.	.	.	.	.	.	-2.4	.	-2.4	.

Numbers indicate log_2-fold changes and colours indicate significant up- (orange) or down-regulation (blue) of genes ($Log_2FC \geq 2$; $p < 0.001$). Treatment abbreviations are composed as female x male from Helgoland (H) and Spitsbergen (S) at 10°C (10) and 20.5°C (20). Two comparisons were omitted for clarity due to low regulation (HxS 10 vs. SxH 10; HxS 20 vs. SxH 20). * denotes contigs which were annotated with the same Uniref ID and could not be confirmed as separate genes.

Table 8 Log_2-fold changes for differentially expressed genes with annotated function in regulating cellular stress responses across lineages and temperature treatments of *Laminaria digitata* sporophytes in experiment 2.

	20°C within lineages			10°C among lineages					20°C crosses		20°C crosses vs. 10°C controls			
sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
control →	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Putative gene product														
Alternative oxidase isoform A	2.4	.	.	.	.	.	.	.	-3.2	.	.	.	.	.
Glutaredoxin	.	.	.	.	.	.	.	.	.	.	.	.	-2.0	-2.1
Heat shock protein 70 *	.	.	.	.	.	.	.	.	.	.	.	.	2.3	.
Heat shock protein 70 *	.	.	.	.	.	.	.	.	.	.	.	.	2.4	.
Heat shock protein 70 *	.	.	.	.	.	.	.	.	.	.	.	.	2.3	.
Heat shock protein 70 *	.	.	.	.	.	.	.	.	.	.	.	.	2.5	.
Peroxidase	-2.1	.	.	.	.	.	.	.	.	.	.	.	.	.
Putative dehydroascorbate reductase	.	.	2.1	.	.	.	.	.	.	.	.	2.3	.	.
Putative respiratory burst oxidase homolog protein	.	.	.	.	.	.	.	.	-5.0	-4.2	.	.	.	.
Putative respiratory burst oxidase homolog protein	.	.	.	.	-2.2	.	.	.	.	.	-4.9	-3.5	.	.
Putative respiratory burst oxidase homolog	.	.	.	.	.	.	.	.	-6.0	-4.2	-3.8	.	.	.
Superoxide dismutase	2.3	.	.	.	.	.	-2.6	-2.2	-4.5	-2.6	.	.	-3.1	.

Numbers indicate log_2-fold changes and colours indicate significant up- (orange) or down-regulation (blue) of genes ($log_2FC \geq 2$; $p < 0.001$). Treatment abbreviations are composed as female x male from Helgoland (H) and Spitsbergen (S) at 10°C (10) and 20.5°C (20). Two comparisons were omitted for clarity due to low regulation (HxS 10 vs. SxH 10; HxS 20 vs. SxH 20). * denotes contigs which were annotated with the same Uniref ID and could not be confirmed as separate genes.

DISCUSSION

In this study, we demonstrated potential beneficial effects of outbreeding among populations within a marine forest key species. Microscopic sporophytes of the inbred Spitsbergen lineage presented the lowest thermal tolerance with substantial decreases in viability at 21 and 22°C. Meanwhile, health of the reciprocal crosses among Helgoland and Spitsbergen *L. digitata* at these temperatures was intermediate to both selfings, but higher than the average selfing response (mid-parent heterosis; Hochholdinger and Hoecker, 2007). In growth and optimum quantum yield of macroscopic sporophytes, both crosses behaved similarly to the inbred Helgoland lineage, while inbred Spitsbergen sporophytes died within seven days at 19 and 20.5°C. Transcriptomic responses revealed that, despite the phenotypic similarity, underlying gene regulation differed among lineages. Gene regulation was often lower in the reciprocal crosses than in the selfings. Among the identified transcripts, some cellular stress responses were reduced. This implies that the reciprocal crosses maintained similar physiology during heat stress with reduced metabolic regulation.

Differentiation in physiological responses among microscopic stages

Gametogenesis and recruitment of juvenile sporophytes at 10°C was significantly faster in the Helgoland selfing than in all other lineages, whereas development was slowest in the Spitsbergen selfing (**Figure 1**). The parthenogenesis controls show that the Helgoland female strain used in this experiment became fertile faster than the Spitsbergen female. Therefore, a potential mismatch in fertilizing speed between males and females of the different locations might have reduced gametogenesis speed in the reciprocal crosses. Gametophytes from both locations readily become fertile between 5 and 15°C (Franke, 2019; Martins et al., 2020), so the experimental temperature of 10°C was likely not stressful for the Spitsbergen material. Müller et al. (2008) interpreted differing capacities for sporophyte formation in a crossing experiment of Arctic and Helgoland *Saccharina latissima* gametophyte strains as a demonstration of ecotypic differentiation. Their experiment, however, showed results reverse to ours, as the Spitsbergen selfing yielded the highest amount of recruited sporophytes at 10°C, while Helgoland females produced the fewest sporophytes. As our experiment (and that of Müller et al., 2008) was conducted only based on four unialgal gametophyte cultures, assumptions on the ecotypic differentiation of the source populations have to be made with caution. However, a recent study has shown differentiation in heat response of *L. digitata* populations across its entire latitudinal distribution range, in which wild sporophytes from Spitsbergen were slightly more sensitive to heat than *L. digitata* from more southern populations (Liesner et al., 2020a), which is supported by our results.

In experiment 1, we identified an upper survival temperature of 21–22°C for microscopic *L. digitata* sporophytes over 14 days (**Figure 2**). This is concordant with the published limits for macroscopic, cultivated *L. digitata* sporophytes (Bolton and Lüning, 1982; tom Dieck, 1992; Franke, 2019; Martins et al., 2019). The Spitsbergen selfing was more sensitive to temperatures ≥ 19°C than the reciprocal crosses and the Helgoland selfing, which is further evidence for differentiation among the Helgoland and Spitsbergen strains of *L. digitata* used in this experiment. However, juvenile macroscopic *L. digitata* sporophytes obtained by fertilizing several different gametophyte isolates from Spitsbergen survived 22°C over two weeks (Franke, 2019). This indicates that the Spitsbergen lineage used in this experiment showed signs of inbreeding depression (Raimondi et al., 2004). Alternatively, this may be an expression high intraspecific plasticity and variation among individuals of the same populations, as was shown for Helgoland *L. digitata* (Liesner et al., 2020b).

Mid-parent heterosis became evident in survival of the reciprocal crosses in experiment 1. Heterosis has been shown to occur in offspring of closely related kelp species (tom Dieck and de Oliveira, 1993; Martins et al., 2019) and may manifest in different ways. Kelp hybrids of *L. digitata* and *L. pallida* showed mid-parent heterosis in optimal growth at 12°C, but had a 2–3°C higher thermal tolerance than either inbred parent (best-parent heterosis; Hochholdinger and Hoecker, 2007). Within a species, outbreeding across populations may alleviate negative effects of genetic drift and inbreeding depression (Charlesworth and Charlesworth, 1987; Raimondi et al., 2004). For *L. digitata*, this may affect especially populations at the species' southern range limit in Brittany, France (Valero et al., 2011). Beneficial effects of outbreeding on growth and fertility have been shown for the giant kelp *Macrocystis pyrifera* (Raimondi et al., 2004; Westermeier et al., 2010) and may be used to produce stable and productive cultivars for mariculture (Westermeier et al., 2010). In contrast, the potential for outbreeding among natural populations is likely limited in seaweeds due to their low dispersal capacity (Norton, 1992; Billot et al., 2003; King et al., 2018).

Gene regulation among lineages of macroscopic sporophytes

In experiment 2, the Helgoland selfing and both reciprocal crosses behaved very similarly apart from small, but significant differences in growth rates and optimum quantum yield F_v/F_m. Only the Spitsbergen selfing was affected by major bleaching after 3 days in all sporophytes at 19 and 20.5°C. This was an extreme reaction and was not expected due to other recent studies showing a conserved upper thermal limit along *L. digitata*'s distribution (Franke, 2019; Liesner et al., 2020a). Therefore, we may suspect that the susceptibility of the Spitsbergen selfing was a result of inbreeding rather than strong local adaptation to the Arctic climate. Compared to the Spitsbergen selfing, outbreeding among populations evidently increased stress resilience as is visible in performance of the reciprocal crosses.

The similar physiology among the surviving lineages in experiment 2 deviates from the different reaction of microscopic stages among lineages in experiment 1. Due to the evidence for differentiation among populations in experiment 1, this evokes the question how the lineages regulated their metabolism to maintain a similar physiological response during heat stress. The differential expression of 3.74% of the 23,288 contigs across lineages in response to temperature is comparable to the regulation of 2.96% of all contigs in *Saccharina latissima* in response to hyposalinity and temperature (Li et al., 2019). However, regulation may be much higher under multiple stressors, as shown by the differential expression of transcripts in *S. latissima* reaching 13% (Heinrich et al., 2015) and even 32% (Heinrich et al., 2016) in response to differing intensities of photosynthetically active radiation (PAR), UV radiation and temperature. In our study, Arctic *L. digitata* shows higher regulation than the cold-temperate Helgoland material at control conditions of 10°C (**Figure 6B**). In contrast, gene regulation in *S. latissima* from the cold-temperate population of Roscoff was double that of Arctic Spitsbergen samples at 8°C (Monteiro et al., 2019b), indicating different regulation patterns across species.

If we interpret the magnitude of gene expression itself as a stress response associated with metabolic costs (Clarke, 2003; Dekel and Alon, 2005; de Nadal et al., 2011), the pattern of reduced gene regulation in the reciprocal crosses compared to the selfings shows a general benefit of outbreeding among individuals from Spitsbergen and Helgoland (**Figure 6B,C**). The contribution of either female or male parents of Helgoland *L. digitata* was sufficient to reduce gene regulation compared to either selfing at 10°C (**Figure 6B**) and compared to the Helgoland selfing at 20°C (**Figure 6C**). A bias towards the female parent response, such as shown for thermal tolerance of interspecific kelp crosses (Martins et al., 2019), could not clearly be identified in the physiological response of intraspecific crosses in our study. However, gene regulation was slightly weaker (i.e. fewer up-regulated and more down-regulated genes) in the outbred lineage with the contribution of a Helgoland female (H x S) compared to the Spitsbergen female (S x H), which is also visible in the divergence of the reciprocal crosses at 20°C in the PCA (**Figure 5**).

Regulated pathways

The functionally annotated genes reflect mostly known pathways in kelp stress responses (see e.g. Heinrich et al., 2012b; Salavarría et al., 2018; Li et al., 2019; Monteiro et al., 2019b). The regulation of genes involved in lipid metabolism indicates that cell and plastid membrane properties were altered in response to heat (**Table 5**). Lipases may have many functions in maintaining lipid homeostasis and structural integrity of membranes (Padham et al., 2007; Zhang et al., 2016). Lipase genes were up-regulated in all lineages in response to heat, but most strongly in the Helgoland selfing. A gene for fatty acid desaturase was down-regulated in response to 20.5°C in all lineages,

mirroring the seasonal decrease of polyunsaturated fatty acids (PUFAs) of several macroalgae in summer (Nelson et al., 2002). As PUFAs and high temperature increase cell membrane fluidity, an increased fraction of unsaturated fatty acids might maintain cell membrane rigidity in response to stress (Neidleman, 1987; Tatsuzawa et al., 1996; Maulucci et al., 2016). Lipoxygenases, on the other hand, oxidize PUFAs and produce oxylipins which act as signaling molecules in development, but also in stress responses (Ritter et al., 2008, 2017; Hou et al., 2015; Maynard et al., 2018). Lipoxygenase genes were down-regulated in response to heat across lineages in this experiment. The up-regulation of acyl-CoA synthetase expression in lineages involving the Spitsbergen female (S x S, S x H) indicates increased catabolism and β-oxidation of stored fatty acids (Blanco and Blanco, 2017). Neutral lipid contents (i.e. storage lipids) in kelp sporophytes are generally low, but may still be mobilized for metabolic processes in the meristem (Haug and Jensen, 1954; Velimirov, 1979). Seasonal patterns show a decrease of lipids from winter to spring in Norwegian *L. digitata* (Haug and Jensen, 1954), which implies a seasonal use as energy storage, which may be induced by increasing temperature.

In addition to cell membrane modification, regulation of genes involved in carbohydrate metabolism revealed structural changes to the cell wall (**Table 6**). The down-regulation of mannuronan C-5 epimerase genes in response to heat in all lineages indicates an increase of cell wall rigidity by structural modification of the major brown algal cell wall component alginate (Nyvall et al., 2003; Dittami et al., 2012; Fischl et al., 2016). Genes related to synthesis of the main cell wall component cellulose (cellulose synthase; Yin et al., 2009) were only down-regulated in the Helgoland selfing at 20.5°C which indicates further structural changes to the cell wall. The down-regulation of a glycosyltransferase gene in response to heat in all lineages may indicate a further reduction in biosynthesis of cell wall polysaccharides (Scheible and Pauly, 2004; Welner et al., 2017; Amos and Mohnen, 2019). However, glycosyltransferases may have various specific functions in glycolipid, glycoprotein and polysaccharide synthesis (Keegstra and Raikhel, 2001), so the actual function remains hypothetical. Related to the assimilation of inorganic carbon, expression of carbonic anhydrase (CA) was up-regulated in all lineages involving Spitsbergen at 10°C compared to the Helgoland selfing. Carbonic anhydrase converts HCO_3^- to CO_2, thereby making it available for carbon assimilation by RuBisCO in a carbon concentrating mechanism (CCM; Raven, 1991; Badger and Price, 1994). Paradoxically, high CA activity was shown for macroalgae from polar environments, where low temperature and salinity allow high concentrations of dissolved CO_2 in the seawater (Wiencke and Fischer, 1990; Gordillo et al., 2006). However, cold temperature may require an increase in cellular enzyme levels, including CA (the "Arctic paradox" of the C_i uptake system; Gordillo et al., 2006). If high CA expression is an adaptive trait of Spitsbergen *L. digitata*, this might have been transferred to both reciprocal crosses. In general carbohydrate metabolism, the up-regulation of endo-1,3-beta-glucanase genes in the reciprocal crosses at 20.5°C compared to the

Helgoland selfing might have increased mobilization of the long-term storage compound laminarin (Chesters and Bull, 1963; Martín-Cuadrado et al., 2008), or is indicative of a pathogen defense to digest fungal or oomycete cell walls (Damasceno et al., 2008; Zhou et al., 2013). The up-regulation of pyruvate kinase expression in the cross-comparison of the reciprocal crosses at 20°C compared to the Spitsbergen selfing at 10°C further indicates increased glycolysis of storage carbohydrates in the crosses (Davison, 1987; Gómez and Huovinen, 2012). These results support a regulation of carbohydrate metabolism in favour of cell maintenance rather than growth, as is visible in the reduced growth rates in experiment 2 (**Figure 3**).

A reduction of chlorophyll biosynthesis (**Table 7**) was indicated in all lineages in response to heat by the down-regulation of genes coding for the key enzymes magnesium chelatase (Pontier et al., 2007; Stenbaek and Jensen, 2010) and protochlorophyllide reductase (Begley and Young, 1989). Regulation of chlorophyll biosynthesis is a known response to several stressors such as hyposalinity, temperature, PAR and UV radiation in the seaweeds *Saccharina latissima* (Heinrich et al., 2015; Monteiro et al., 2019b) and *Desmarestia anceps* (Iñiguez et al., 2017). Whereas growth at sublethal temperature may increase chlorophyll biosynthesis in kelps (Li et al., 2019), the reduction in our study may be interpreted as a heat stress response (Tewari and Tripathy, 1998). Monogalactosyldiacylglycerol (MGDG) synthase expression was generally up-regulated in the reciprocal crosses, indicating maintenance of photosynthetic function as MGDG is a dominant glycolipid in thylakoid membranes (Kobayashi et al., 2004). Further, genes involved in synthesis of photosystem components (HCF101; thylakoid lumenal 15kDa protein; photosystem I reaction center subunit XI; light harvesting complex protein; Lezhneva et al., 2004; Liu et al., 2012) were regulated in unclear patterns. Only a gene coding for a Rieske (2Fe-2S) domain containing protein was up-regulated in all lineages involving Spitsbergen in comparison to the Helgoland selfing, indicating higher investment in electron transport (Malkin and Posner, 1978; De Vitry et al., 2004). In *S. latissima*, genes facilitating production of photosystems, thylakoid membranes and LHC proteins were uniformly up-regulated at 2°C as an acclimation reaction to cold temperature (Heinrich et al., 2012b). Reduced expression of a violaxanthin de-epoxidase (VDE) gene may either indicate a reduction of nonphotochemical quenching of excessive energy via the xanthophyll cycle in response to heat (Goss and Jakob, 2010; Latowski et al., 2011) or reduced synthesis of light-harvesting carotenoids (Dautermann et al., 2020). De-epoxidation of xanthophyll cycle pigments has recently been shown as a heat response in several *L. digitata* populations, among which Helgoland showed the weakest response, indicating that VDE activity may be population specific (Liesner et al., 2020a). The stability of optimum quantum yield at 20.5°C in this study suggests that regulation of the above genes is a means of maintaining photosynthetic efficiency even under high thermal stress (**Figure 4**).

Oxidative stress occurs when the homeostasis among pathways is disturbed by stressors (e.g. high temperature disturbing the photosynthetic electron transport chain), leading to the release of reactive oxygen species (ROS; Suzuki and Mittler, 2006). Some typical genes in response to oxidative stress (Peroxidase; dehydroascorbate reductase; Tang et al., 2006; Do et al., 2016) were differentially expressed only in single comparisons (**Table 8**). The expression of superoxide dismutase is a known heat stress response in kelps (Heinrich et al., 2012b; Yotsukura et al., 2012), which, according to our results, is reduced in outbred lineages of *L. digitata*. The down-regulation of genes coding for respiratory burst oxidase homolog proteins in the reciprocal crosses at 20.5°C compared to the Helgoland selfing further indicate a reduced stress response in the crosses. Oxidative burst (i.e. the active production of ROS; Bhattacharjee, 2005) may be a defense reaction to pathogens or grazing triggered by degradation products of cell wall alginates (Cosse et al., 2007; Bischof and Rautenberger, 2012; Ritter et al., 2017). However, ROS may also be used in stress-related or developmental signalling cascades (Miller et al., 2008; Pearson et al., 2019). Reduced oxidative stress response is also visible in the reduced expression of genes for alternative oxidase (Vanlerberghe, 2013) and glutaredoxin (Laporte et al., 2012) in the reciprocal crosses. The chaperone heat shock protein 70 (HSP70; Wang et al., 2004) was differentially expressed only in the H x S cross at 20.5°C compared to the Spitsbergen selfing at 10°C. The temperature eliciting maximum HSP70 expression differed among British *L. digitata* populations, and may be a distinct, population-specific temperature between 16–24°C (King et al., 2019). This indicates that the lineages tested in this study might have differed in the temperature evoking the maximum HSP70 response. Further, HSPs are part of a short-term reaction (e.g. 1 h heat shock in King et al., 2019), whereas they are reduced in longer term acclimation (Heinrich et al., 2015; Monteiro et al., 2019b). Surprisingly and in contrast to other abiotic stress studies on kelps, we could not identify vanadium-dependent haloperoxidases (Roeder et al., 2005; Salavarría et al., 2018; Li et al., 2019; Monteiro et al., 2019b), which are essential enzymes in the response to oxidative stress (Bischof and Rautenberger, 2012).

Implications of outbreeding for mariculture and marine forest conservation

Our results imply that outbreeding among distant kelp populations may mitigate effects of genetic drift and may increase stress tolerance in comparison to inbred lineages. As shown by the reduced gene regulation in the reciprocal crosses at thermal stress, performance during heat stress may even improve by outbreeding with an Arctic population. This adds to the extensive knowledge from terrestrial agriculture and recently also kelp mariculture, that inter-cultivar crosses may produce stable, healthy descendants with superior characteristics compared to their inbred parental lineages (Li et al., 2007; Westermeier et al., 2010; Fu et al., 2014). In kelps, the relatively simple production and maintenance of stable gametophyte stock cultures provides a solid foundation to explore kelp cultivation and breeding programs (Westermeier et al., 2010; Bartsch, 2018). However, differences

in phenotypic plasticity among kelp individuals (Mabin et al., 2019; Liesner et al., 2020b) may result in substantial response variation regardless of whether cultivars were in- or outbred (Camus et al., 2018). Therefore, to produce stable phenotypes and maximize heterosis, homozygous lines should be inbred over several generations before application in mariculture (Robinson et al., 2013).

Meanwhile, natural kelp populations are threatened by ocean warming especially at the warm range edges (Voerman et al., 2013; Wernberg et al., 2016; Smale et al., 2019). The southernmost *L. digitata* populations in France are predicted to collapse until the end of the century under the worst-case climate change scenario (Raybaud et al., 2013; Assis et al., 2018). Currently, these populations harbour high and potentially unique genetic diversity due to their potential persistence across glacial cycles (Assis et al., 2018; Liesner et al., 2020a; Neiva et al., 2020). In addition to gradual ocean warming, increasingly intense and frequent marine heatwaves (Hobday et al., 2016; Oliver et al., 2018) may reduce genetic diversity of persistent populations (Coleman et al., 2020a; Gurgel et al., 2020). A cautious method for conservation of threatened natural populations may therefore be an introduction of intraspecific cultivars from distant populations in an attempt of "assisted evolution" (Filbee-Dexter and Smajdor, 2019; Coleman et al., 2020b).

An important caveat in this tentative proposal is the potential for outbreeding depression in crosses of locally adapted populations, which reduces performance of crossed lineages (McKay et al., 2005; Aitken and Whitlock, 2013). As yet, outbreeding depression could not be identified among distant populations of the fucoid seaweed *Hormosira banksii* (McKenzie and Bellgrove, 2006), and was also not obvious in performance of the reciprocal crosses here. This suggests that genetic divergence among *L. digitata* populations is low, despite the strong spatial structuring and thermal gradient along the species' distribution (Liesner et al., 2020a). Alternatively, outbreeding depression my manifest later in the life cycle especially with respect to reproduction (Schierup and Christiansen, 1996; McKenzie and Bellgrove, 2006). Therefore, careful assessments of the performance and viability of numerous outbred lineages are necessary on large time scales before considering an application in natural populations. Ultimately, we have to face the ethics of interfering with natural systems (Filbee-Dexter and Smajdor, 2019). Anthropogenic climate change alters natural environments, but what we categorize as "natural" (e.g. pre-industrial conditions) cannot be restored by assisted evolution. We can only deliberately modify ecosystems to persist in a changing environment (Filbee-Dexter and Smajdor, 2019; Coleman et al., 2020b). Therefore, we have to carefully assess why and to what extent we should interfere with the natural reaction of ecosystems to "unnatural" anthropogenic climate change.

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Data availability statement

The Illumina sequence reads generated during this study have been submitted to the NCBI Sequencing Read Archive (SRA) under the accession PRJNA665130.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1 Log_2-fold changes for differentially expressed genes with annotated function across lineages and temperature treatments of *Laminaria digitata* sporophytes.

	20°C within lineages			10°C among lineages					20°C crosses		20°C crosses vs. 10°C controls			
sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
control →	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Putative gene product														
26S proteasome regulatory subunit	.	.	2.0	.	.	.	.	.	.	.	.	2.0	.	.
5-hydroxyisourate hydrolase	.	.	.	.	.	.	.	.	.	.	.	2.2	.	.
Acetyl-CoA acetyltransferase	.	.	.	.	.	.	.	.	.	.	.	2.2	.	.
Acyl-CoA synthetase	.	.	.	.	.	.	.	.	.	.	.	2.1	.	.
Acyl-CoA synthetase	.	.	.	.	.	.	.	.	.	.	.	2.0	.	.
Acyl-CoA synthetase	.	.	.	2.0	.	.	.	.	.	.	.	2.2	.	.
Agmatinase	.	.	.	.	.	.	.	.	-2.3	.	.	.	.	.
Alpha-(1,6)-fucosyltransferase, family GT23	.	.	.	5.4	.	.	.	.	.	.	.	.	.	.
Alternative oxidase isoform A	2.4	.	.	.	.	.	.	.	-3.2	.	.	.	.	.
Ankyrin (Fragment)	-2.1	.	.	.	.	.	.	.	.	.	.	.	.	.
Asn/thr-rich large protein family protein	.	.	.	.	.	.	.	.	.	.	.	-2.5	.	.
ATP-binding cassette superfamily	.	.	.	.	.	.	.	.	-3.1	.	.	.	.	.
ATP-dependent Clp protease proteolytic subunit	.	.	.	.	.	.	.	.	.	.	-2.1	-2.0	-2.1	-2.0
Beta-lactamase domain protein	5.8	.	.	.	.	.	.	.	-3.1	-3.1	.	.	.	.
Beta-lactamase domain protein	.	.	.	5.4	.	.	.	.	.	.	.	.	.	.
Beta-lactamase domain protein	6.3	.	3.7	.	.	.	.	.	-3.7	-2.9	.	.	.	.
Binding	.	.	-2.2	.	.	.	.	.	.	.	-2.7	-2.8	.	.
Carbonic anhydrase	.	.	.	3.4	2.4	2.6	.	.	.	.	.	2.0	.	.
Cellulose synthase (UDP-forming), family GT2	-2.5	.	.	.	.	.	.	.	.	.	.	.	.	.
Cellulose synthase (UDP-forming), family GT2	-2.3	.	.	.	.	.	.	.	.	.	.	.	.	.

Supplementary Table 1 (continued)

	20°C within lineages			10°C among lineages					20°C crosses		20°C crosses vs. 10°C controls			
sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
control →	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Putative gene product														
Chp-1 / RAR1 homologue	2.4	.	.	.	.	.	.	.	.	.	.	2.2	.	.
COX10 homolog, cytochrome c oxidase assembly protein	.	.	.	.	.	.	.	.	.	.	.	2.2	.	.
Cyclin A-like protein	.	.	.	.	.	.	.	.	-2.5	.	.	.	.	.
Cyclin B2	.	-2.2	-2.2	.	.	.	.	.	.	.	.	.	-2.7	-2.7
Cysteine desulfuration protein	.	.	.	.	.	.	.	.	.	.	.	2.1	.	.
Cytochrome P450	.	-2.3	.	.	.	.	.	.	.	.	.	.	.	.
D-tyrosyl-tRNA(Tyr) deacylase	2.6	2.6	3.1	.	.	.	.	.	.	.	2.7	3.3	.	2.4
Elongation of fatty acids protein	.	.	.	.	.	.	.	.	.	.	.	.	-2.2	-2.1
Endo/excinuclease amino terminal domain-containing pr.	.	.	.	.	.	.	.	.	.	.	.	2.1	.	.
Endo-1,3-beta-glucanase, family GH81	.	4.1	.	.	.	.	.	.	6.0	.	6.3	4.8	.	.
Endo-1,3-beta-glucanase, family GH81	.	3.5	.	.	.	.	.	.	5.7	.	6.1	4.6	.	.
EsV-1-12	.	.	.	5.1	.	.	.	.	.	.	4.7	.	.	.
EsV-1-163	.	.	2.1	-5.8	.	.	.	.	.	.	.	.	6.3	6.5
EsV-1-166	.	.	.	.	.	.	.	.	.	.	4.1	.	.	.
FAD linked oxidase domain-containing protein	5.2	.	.	.	.	.	.	.	-5.1	-3.8	.	.	.	.
Fatty acid desaturase	-2.2	-2.0	-2.1	.	.	.	.	.	.	.	.	-2.1	.	.
Ferredoxin nitrite reductase	-2.5	.	.	.	.	.	.	.	.	.	.	.	.	.
Fibronectin type III domain protein	.	.	.	.	.	.	.	.	.	.	-2.2	.	-2.1	.
FirrV-1-B30	.	.	.	.	.	.	.	.	.	.	.	.	.	-2.3
FirrV-1-B30	-3.1	.	-3.6	.	.	.	.	.	.	.	.	-2.1	.	-2.8
Flagellar associated protein, quinone reductase-like protein	.	.	.	.	.	.	.	.	.	.	.	2.3	.	.
Flagellar outer arm dynein 14 kDa light chain LC5	-2.3	.	.	.	.	.	.	.	.	.	.	.	.	.
FRIGIDA-like protein 3	-3.7	-2.2	-2.1	.	.	.	.	.	.	.	.	-2.1	.	.

Supplementary Table 1 (continued)

	20°C within lineages			10°C among lineages					20°C crosses		20°C crosses vs. 10°C controls			
sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
control →	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Putative gene product														
Gal-2,6-Sulfurylases I	.	2.1	.	.	.	.	.	.	.	.	2.0	.	.	.
Genome assembly	.	.	2.1	.	.	.	.	.	.	.	.	.	.	.
Genomic scaffold, scaffold_80	.	.	.	3.9	.	.	-5.1	-3.3	.	.	.	.	.	-3.8
Genomic scaffold, scaffold_80	.	.	.	4.0	.	.	-4.2	-2.8	.	.	.	.	.	-3.2
Glutaredoxin	.	.	.	.	.	.	.	.	.	.	.	.	-2.0	-2.1
Glycin-rich protein	.	4.0	2.8	.	.	.	.	.	.	.	5.3	5.8	4.9	5.6
Glycin-rich protein	4.7	3.7	3.2	.	.	.	.	.	.	.	6.0	6.6	6.8	7.3
Glycosyltransferase, family GT4	-2.8	-3.1	-2.4	.	.	.	.	.	.	.	-2.7	-2.7	-2.5	-2.5
Haloacid dehalogenase-like hydrolase family protein	.	.	2.0	.	.	.	.	.	.	.	.	2.4	.	.
HCF 101; ATP binding	.	.	2.3	.	.	.	.	.	.	.	.	2.1	.	.
Heat shock protein 70	.	.	.	.	.	.	.	.	.	.	.	.	2.3	.
Heat shock protein 70	.	.	.	.	.	.	.	.	.	.	.	.	2.4	.
Heat shock protein 70	.	.	.	.	.	.	.	.	.	.	.	.	2.3	.
Heat shock protein 70	.	.	.	.	.	.	.	.	.	.	.	.	2.5	.
Hemolysin-type calcium-binding region	.	.	.	.	.	.	.	.	.	.	3.8	.	3.8	.
Hemolysin-type calcium-binding region	.	.	.	-2.4	.	.	.	.	.	.	-2.3	.	-2.7	.
Hypothetical leucine rich repeat kinase	.	.	.	-7.8	.	.	7.3	7.4	.	.	.	.	9.0	8.7
Hypothetical leucine rich repeat protein	-2.9	.	.	.	.	.	.	.	.	.	.	.	.	.
Hypothetical leucine rich repeat protein	.	-5.3	.	7.2	6.1	5.5	.	.	.	.	.	3.3	-7.1	-2.3
Ig-like protein, group 2	.	.	.	.	.	.	.	.	-3.1	.	-3.1	.	-2.2	.
Imm upregulated 13	.	3.2	.	.	.	.	.	.	.	.	5.5	.	.	.
Imm upregulated 3	.	3.5	.	.	.	.	.	.	.	.	5.6	.	.	.
Imm upregulated 3	3.1	.	.	.	.	.	.	.	.	.	.	.	.	.

Supplementary Table 1 (continued)

	20°C within lineages			10°C among lineages					20°C crosses		20°C crosses vs. 10°C controls			
sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
control →	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Putative gene product														
Imm upregulated 3	.	.	.	.	.	.	.	.	.	.	.	4.2	.	.
Imm upregulated 3	.	.	.	-7.2	.	.	6.1	6.0	.	.	.	.	6.8	6.7
Immunophilin	.	-2.0	.	.	.	.	.	.	.	.	-2.5	-2.0	.	.
Light harvesting complex protein	.	.	.	.	.	.	.	.	.	.	2.0	2.2	.	.
Lipase	4.5	.	.	.	.	.	.	.	-5.0	-3.5	.	.	.	.
Lipase	2.7	.	.	4.5	3.3	3.8	.	.	.	.	4.2	3.8	.	.
Lipase	2.3	3.0	3.5	.	.	.	.	.	.	.	.	.	5.4	5.3
Lipoxygenase	-3.4	.	.	.	.	.	.	.	-5.4	-3.8	-3.4	.	-3.1	.
Lipoxygenase	-3.4	.	.	.	.	.	.	.	-5.2	-3.6	-3.4	.	-2.4	.
Long-chain acyl-CoA synthetase	.	.	2.0	.	.	.	.	.	.	4.6	.	6.0	.	.
LRR-GTPase of the ROCO family	.	.	.	6.1	.	.	.	.	.	.	5.2	4.7	.	.
LRR-GTPase of the ROCO family	.	.	.	-3.9	.	.	.	.	.	.	.	.	.	.
Magnesium chelatase subunit H, putative chloroplast	-2.8	-2.3	-2.2	.	.	.	.	.	.	.	.	-2.2	.	.
Magnesium chelatase subunit H, putative chloroplast	-3.3	-2.1	-2.3	.	.	.	.	.	.	.	.	-2.6	.	-2.1
Magnesium-protoporphyrin IX methyltransferase, putative chloroplast	-2.3	.	.	.	.	.	.	.	.	.	.	.	.	.
Mannuronan C-5 epimerase	-3.7	-3.7	-4.3	.	.	.	.	.	.	.	-2.7	-3.2	-3.1	-3.7
Mannuronan C-5-epimerase	-2.7	.	.	.	.	.	.	.	.	.	.	.	-2.1	-2.1
Mannuronan C-5-epimerase	-3.5	-2.4	-2.6	.	.	.	.	.	.	.	-2.3	-2.9	.	-2.4
Mannuronan C-5-epimerase N-terminal	.	-4.2	-3.3	.	.	.	.	.	.	.	-5.1	-4.7	-4.5	-4.0
Mar14 transposase	.	.	.	6.2	.	.	.	.	6.0	5.8	6.2	6.7	.	.
Metallophosphoesterase	.	.	.	.	-2.3	.	.	.	.	.	-2.1	-2.3	.	.
Monogalactosyldiacylglycerol synthase, family GT28	.	3.7	3.8	.	.	.	.	.	4.8	5.1	5.3	5.9	2.5	3.1

Supplementary Table 1 (continued)

	20°C within lineages			10°C among lineages					20°C crosses		20°C crosses vs. 10°C controls			
sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
control →	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Putative gene product														
NADPH:adrenodoxin oxidoreductase, mitochondrial	.	.	2.4	.	.	.	.	.	.	.	.	2.5	.	.
Nitrite reductase (NAD(P)H) large subunit	.	.	-2.3	.	.	.	.	.	.	.	.	.	.	-2.0
Nitrite reductase (NAD(P)H) large subunit	.	.	-2.2	.	.	.	.	.	.	.	.	.	.	.
Nitrite reductase (NAD(P)H) large subunit	.	.	-2.3	.	.	.	.	.	.	.	.	.	.	.
Nonribosomal peptide synthetase 10	.	.	.	.	.	.	.	.	.	.	.	.	2.4	.
Nonribosomal peptide synthetase 10	.	.	.	.	.	.	.	.	.	.	.	.	2.2	.
Oxidoreductase domain protein	.	.	2.5	.	.	.	.	.	.	.	2.3	2.9	.	.
PArp-RdRp (Fragment)	.	.	.	.	.	.	.	.	.	.	.	4.3	.	.
Peptidyl-Asp metallopeptidase. MEROPS family M72	2.0	.	.	.	.	.	.	.	-3.8	.	.	.	.	.
Peptidyl-prolyl cis-trans isomerase	.	.	2.5	.	.	.	.	.	.	.	.	2.4	.	.
Peroxidase	-2.1	.	.	.	.	.	.	.	.	.	.	.	.	.
Photosystem I reaction center subunit XI	.	.	.	.	.	.	.	.	.	.	.	.	-4.2	-3.6
Plasma membrane iron permease	-3.4	.	.	.	.	.	.	.	.	2.0	.	.	.	.
Polymorphic outer membrane protein	.	.	.	-2.4	.	.	.	.	.	.	.	.	.	.
Polymorphic outer membrane protein	.	.	.	-2.9	-2.2	.	.	.	.	.	-4.5	-2.6	.	.
Polymorphic outer membrane protein	.	.	.	.	.	.	.	.	.	.	-6.0	.	.	.
Polymorphic outer membrane protein	.	.	.	.	.	.	.	.	.	.	-6.0	-2.8	.	.
PREDICTED: RNA-binding protein Nova-2, partial	.	.	.	.	.	.	.	.	-5.4	-3.7	.	.	.	.
Probable fusion protein	.	.	.	.	.	.	.	.	.	.	.	.	-2.1	.
Probable high CO2 inducible periplasmic protein	-2.1	.	.	.	.	.	.	.	.	.	.	.	.	.
Protein C10orf22, putative	.	.	.	.	.	.	.	.	.	.	.	.	-2.9	-2.4
Protein yippee-like	2.3	.	.	.	.	.	.	.	-2.9	.	.	.	.	.
Proteinase inhibitor I35 domain containing protein	5.4	.	3.9	5.7	.	.	-5.6	-4.5	.	.	.	5.4	.	.

Supplementary Table 1 (continued)

	20°C within lineages			10°C among lineages					20°C crosses		20°C crosses vs. 10°C controls			
sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
control →	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Putative gene product														
Protochlorophyllide reductase, putative chloroplast	-2.8	-2.5	.	.	.	.	.	.	.	.	-2.3	.	-2.3	.
Protochlorophyllide reductase, putative chloroplast	-3.0	.	.	.	.	.	.	.	.	.	.	-2.0	.	.
Putative dehydroascorbate reductase	.	.	2.1	.	.	.	.	.	.	.	.	2.3	.	.
Putative growth-on protein GRO10	.	.	.	3.5	.	.	.	.	.	.	.	.	.	.
Putative respiratory burst oxidase homolog	.	.	.	.	.	.	.	.	-6.0	-4.2	-3.8	.	.	.
Putative respiratory burst oxidase homolog protein	.	.	.	.	.	.	.	.	-5.0	-4.2	.	.	.	.
Putative respiratory burst oxidase homolog protein	.	.	.	.	-2.2	.	.	.	.	.	-4.9	-3.5	.	.
Putative sodium calcium exchanger	-2.1	.	.	.	.	.	.	.	.	.	.	.	.	.
Putative nosD copper-binding protein	.	.	-2.3	.	.	.	.	.	.	.	.	.	.	.
Pyruvate kinase	.	.	.	.	.	.	.	.	.	.	.	.	2.5	2.6
Rab11B, RAB family GTPase	.	.	.	.	.	.	.	.	.	.	.	2.0	.	.
Regulator of G-protein signaling 2	-2.1	.	.	-3.0	.	.	.	2.1	.	.	-2.0	.	.	.
Rieske (2Fe-2S) region	.	.	.	6.8	6.0	5.7	.	.	6.4	4.9	5.7	4.8	.	.
RxLR effector candidate protein	.	4.5	.	.	.	.	.	.	.	.	.	4.2	.	.
Serine O-acetyltransferase	.	.	.	.	.	.	.	.	-2.7	.	.	.	.	.
Serine/threonine protein kinase, possibly NIMA-like	2.0	.	.	.	.	.	.	.	.	.	.	.	.	.
Serine/threonine-protein phosphatase 2A activator	.	.	2.2	.	.	.	.	.	.	.	.	2.0	.	.
Similar to collagen, partial	.	4.8	5.1	.	.	.	.	.	3.0	3.3	4.7	5.0	5.5	5.8
Similar to collagen, partial	.	4.7	4.9	.	.	.	.	.	3.0	3.2	4.5	4.8	5.8	6.0
Similar to DEK oncogene (DNA binding)	.	.	.	.	.	.	.	.	.	.	.	.	-2.8	-2.1
Similar to Patched domain-containing protein 3, partial	2.0	.	.	.	.	.	.	.	-4.3	-3.2	.	.	.	.
Similar to Patched domain-containing protein 3, partial	.	.	.	.	-2.1	.	.	.	.	.	-2.3	-2.5	.	.
Similar to Werner syndrome protein	.	.	.	-4.9	.	.	.	.	.	.	.	.	.	.

Supplementary Table 1 (continued)

	20°C within lineages			10°C among lineages					20°C crosses		20°C crosses vs. 10°C controls			
sample →	H x H 20	H x S 20	S x H 20	S x S 10	H x S 10	S x H 10	H x S 10	S x H 10	H x S 20	S x H 20	H x S 20	S x H 20	H x S 20	S x H 20
control →	H x H 10	H x S 10	S x H 10	H x H 10	H x H 10	H x H 10	S x S 10	S x S 10	H x H 20	H x H 20	H x H 10	H x H 10	S x S 10	S x S 10
Putative gene product														
Singapore isolate B (sub-type 7), scaffold_1	.	.	.	.	.	.	.	.	.	.	.	2.1	.	.
Stackhouse genomic scaffold, scaffold_1	.	.	.	2.3	2.5	3.8	.	.	2.8	.	3.5	2.0	.	.
Superoxide dismutase	2.3	.	.	.	.	.	-2.6	-2.2	-4.5	-2.6	.	.	-3.1	.
Thioesterase	.	.	.	.	.	.	.	.	.	.	.	.	2.3	.
Thioredoxin-like protein	.	.	.	.	.	.	.	.	.	.	.	.	-2.6	.
Thylakoid lumenal 15 kDa protein, chloroplast (P15)	.	.	.	.	.	.	.	.	.	.	.	2.1	.	.
TKL family protein kinase/ putative CTR1-like	.	.	.	.	.	.	.	.	.	.	-3.2	-2.6	.	.
TKL family protein kinase/ putative CTR1-like	.	.	.	.	.	.	.	.	-2.2	.	-2.1	.	.	.
Transmembrane receptor kinase	.	.	.	.	.	.	.	.	.	.	-2.5	-2.7	.	.
transposase mariner transposase undefined product (IC)	.	.	.	.	.	.	.	.	.	.	.	-2.3	.	.
tRNA pseudouridine synthase	.	.	2.0	.	.	.	.	.	.	.	.	2.3	.	.
tRNA pseudouridine synthase	.	.	.	.	.	.	.	.	.	.	.	2.6	.	.
TTK-like	.	.	.	-5.2	.	.	.	.	.	.	.	.	.	.
TTK-like	.	.	.	-4.8	.	.	.	.	.	.	.	.	.	.
Unsaturated glucuronyl hydrolase, family GH88	.	.	.	.	.	.	.	.	-3.1	.	-2.5	.	-2.3	.
Urea/Na+ high-affinity symporter	.	-3.0	-4.6	.	.	.	.	.	.	.	.	-3.0	-3.3	-5.2
VDE domain-containing protein	-2.2	-2	.	.	.	.	.	.	.	.	-2.1	.	.	.
VDE domain-containing protein	-2.4	-2.2	.	.	.	.	.	.	.	.	-2.4	.	-2.3	.
VDE domain-containing protein	-2.4	-2.4	.	.	.	.	.	.	.	.	-2.4	.	-2.4	.
YeeE/YedE family protein	.	.	.	.	.	.	.	.	.	.	.	-2.3	.	.

Numbers indicate $\log_2$-fold changes and colours indicate significant up- (orange) or down-regulation (blue) of genes ($\log_2 FC \geq 2$; $p < 0.001$). Treatment abbreviations are composed as female x male from Helgoland (H) and Spitsbergen (S) at 10°C (10) and 20.5°C (20). Two comparisons were omitted for clarity due to low regulation (HxS 10 vs. SxH 10; HxS 20 vs. SxH 20).

6 Synoptic discussion

6.1 Major findings

Decades ago, general thermal response curves of polar, temperate and tropical seaweed species (e.g. Bolton and Lüning, 1982; Yarish et al., 1986; Orfanidis, 1993; Wiencke et al., 1994; Pakker et al., 1995) and first evidence for thermal ecotypes were published (e.g. Novaczek et al., 1990; Breeman and Pakker, 1994; Martinez, 1999). These studies were primarily conducted to relate the species' thermal characteristics to their biogeographical distribution patterns. Meanwhile, recent research on thermal responses of seaweeds, which builds on this fundamental knowledge, is often motivated by assessing and predicting the effects of climate change (e.g. Hargrave et al., 2017; Nepper-Davidsen et al., 2019; Zacher et al., 2019; Graba-Landry et al., 2020). Such recent studies have uncovered parts of a complex framework of genetic and non-genetic processes driving trait variability within seaweed species (e.g. Mabin et al., 2013, 2019; King et al., 2018; Clark et al., 2020; Supratya et al., 2020). Within this framework, the overarching objective of this dissertation was to produce a detailed assessment of the thermal trait variability of a kelp across populations and life cycle stages using the example of *Laminaria digitata*.

The studies presented in this dissertation contribute to the growing knowledge of trait variation and plasticity in seaweeds. I demonstrated that thermal responses within the kelp *Laminaria digitata* differ among populations, among individuals, across ontogeny and development within individuals, and among inbred and outbred lineages of distant populations. However, my results also underline the adaptation of *L. digitata* as a eurythermal, cold-temperate to Arctic species, whose performance, albeit variable and plastic, is confined within the thermal limits described almost 40 years ago (Bolton and Lüning, 1982). In the style of the theoretical concepts of thermal trait variability within a species which I introduced earlier (**Figure 1.5** – **Figure 1.8**), the main findings of this dissertation are conceptualized in the comprehensive **Figure 6.1**. This figure illustrates the four interactive levels of thermal trait variability for *L. digitata* derived from the three publications and a Master's thesis (Gauci, 2020; see **Chapter 6.1.3**) which were produced within the scope of this dissertation. In this chapter, I lead through the main results summarized in **Figure 6.1** and provide an integrative discussion of the mechanisms shaping thermal trait variability in kelp.

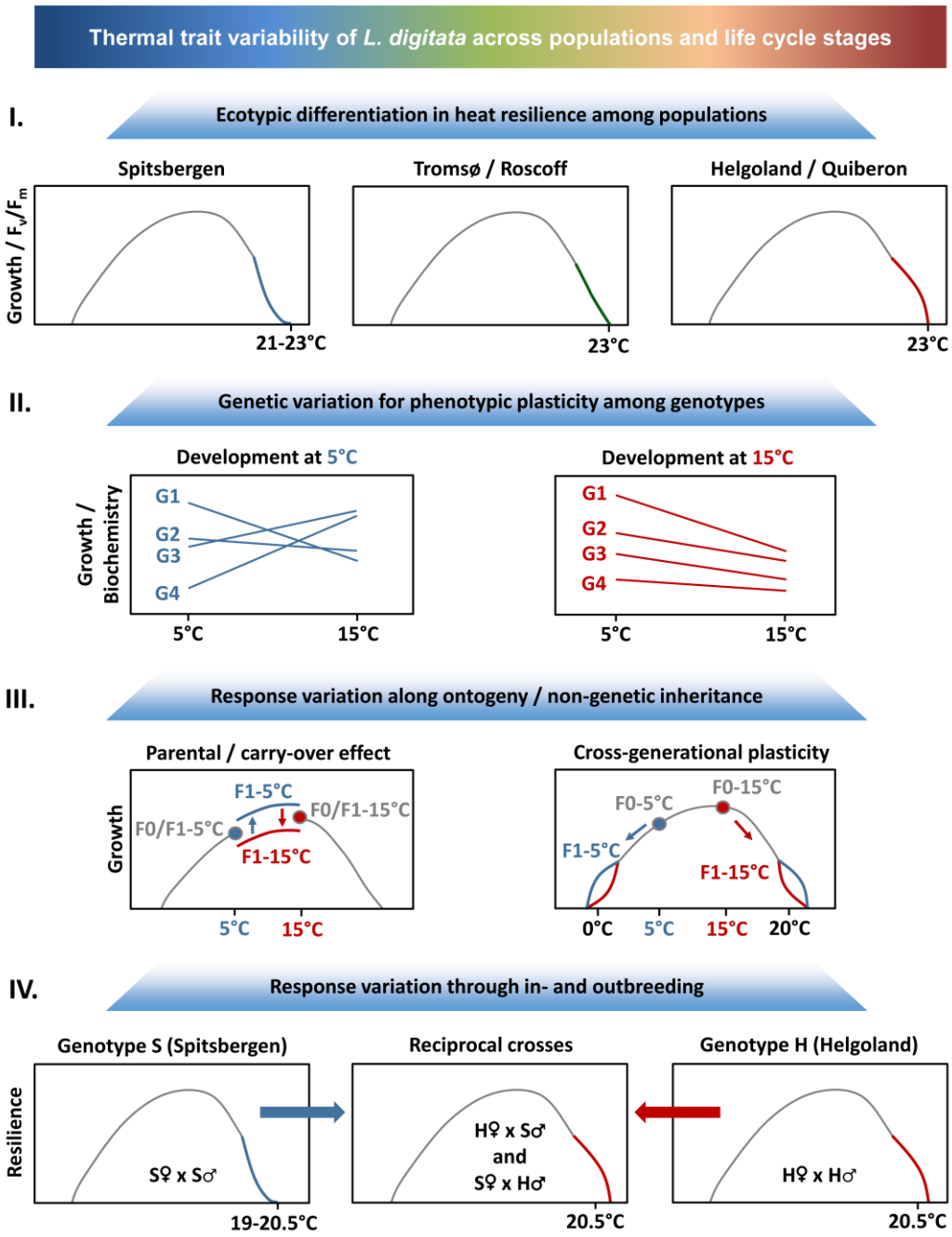

Figure 6.1 Thermal trait variability of *Laminaria digitata* identified in this thesis. Grey reaction norms represent the average species response across a temperature gradient (e.g. thermal growth curves by Bolton and Lüning, 1982; tom Dieck, 1992), coloured lines simplify data generated for this dissertation. **I. Ecotypic differentiation**: Growth and optimum quantum yield (F_v/F_m) showed higher heat sensitivity in the population of Spitzbergen (blue) and higher heat resilience in the populations of Helgoland and Quiberon (red), while heat tolerance was intermediate for Tromsø and Roscoff samples (green; **Publication I**). **II. Genetic variation for phenotypic plasticity**: Reaction norm slopes of growth and biochemical traits differed among genotypes (G) of juvenile sporophytes reared at 5°C (**Publication II**). **III. Parental / carry-over effect**: Juvenile sporophytes (F1; coloured lines) grew faster following treatments of gametogenesis and rearing (F0/F1; grey font, coloured dots) at 5°C (blue) compared to 15°C (red; **Publication II**). **Cross-generational plasticity**: Juvenile sporophytes (F1; coloured lines) grew better only at 0 and 20°C when they were recruited from gametophytes (F0; grey font, coloured dots) pre-cultivated for three years at 5°C (blue) compared to 15°C (red), while growth at 5–15°C was similar among pre-treatments (grey line; Gauci, 2020; **Chapter 6.1.3**). **IV. Inbreeding**: Thermal tolerance differed among sporophytes obtained by inbreeding genotypes from Spitsbergen (S; < 19°C) and Helgoland (H, > 20.5°C). **Heterosis**: Reciprocal crosses among populations were physiologically similar to the Helgoland selfing, but presented lower differential gene expression during heat stress (**Publication III**).

6.1.1 Thermal ecotypes along the latitudinal distribution

Publication I and **Publication III** demonstrate again the high thermal acclimation capacity of *L. digitata* to short-term exposure of up to 21°C, whereas 23°C posed a general growth limit (Bolton and Lüning, 1982; tom Dieck, 1992). However, subtle differences among populations (simplified in **Figure 6.1-I**) contribute to the growing evidence for local adaptation among kelp populations (reviewed by King et al., 2018). In **Publication I**, the southernmost *L. digitata* population of Quiberon, France, showed a slightly higher thermal tolerance compared to the more northern populations in growth at 19°C and recovery from 21°C. The *L. digitata* population of Helgoland, which persists at the species' upper thermal limit in summer (Bartsch et al., 2013), showed reduced photoprotective responses and only slight reduction of optimum quantum yield even at 23°C, indicating high heat resilience. In contrast, *L. digitata* sporophytes from the distributional leading edge on the Arctic archipelago of Spitsbergen had the strongest negative response to 21 and 23°C visible in strong reduction of F_v/F_m (**Publication I**) and in reduced survival at presumed sublethal temperatures of 19–21°C (inbred sporophytes in **Publication III**; one meristem sample in **Publication I**). Potentially, a lack of selection pressure for heat tolerance might have led to a reduction of function at high temperature for Arctic *L. digitata* (Wiencke et al., 1994; Lahti et al., 2009). Differences in overall transcriptomic profiles suggested substantial differentiation in gene regulation among individuals from the populations of Helgoland and Spitsbergen as became visible in the principal component analysis (**Figure 5** in **Publication III**). The number of differentially expressed genes suggests that metabolic costs at the species' presumed optimum temperature of 10°C (Bolton and Lüning, 1982; tom Dieck, 1992) were higher for inbred Spitsbergen sporophytes than for Helgoland sporophytes despite their similar physiological response (**Publication III**). Neutral microsatellite markers revealed strong hierarchical structuring among populations, which is confirmed by other recent publications (**Publication I**; King et al., 2020a; Neiva et al., 2020). Hierarchical structuring of extant *L. digitata* populations is assumed to be the result of persistence in distinct glacial refugia during the Last Glacial Maximum (LGM; Clark et al., 2009), after which genetically distinct populations expanded and recolonized the North Atlantic (Neiva et al., 2020). The phylogeographic separation into a northern and a southern Northeast Atlantic clade might have facilitated the divergence of *L. digitata* populations across glacial cycles.

However, physiological differentiation was evident only at the species' ecological range edges. In contrast, the two populations of Roscoff, France, and Tromsø, Norway, among which average yearly temperatures differ by several degrees (6.2°C in 2018; **Publication I)**, did not differ significantly in their responses of F_v/F_m and xanthophyll pigments, which had revealed the differentiation among the populations of Spitsbergen and Helgoland. Therefore, local adaptation may not be explained by the thermal regime alone. Greater population connectivity and reduced selective pressure might hinder local adaptation towards a species' range centre (Eckert et al., 2008; Hardie and Hutchings, 2010).

Alternatively, local adaptation may become evident in different traits affecting fitness which I did not assess (e.g. traits related to reproduction).

My results contribute to the recently growing evidence of ecotypic differentiation among kelp populations (e.g. Olischläger et al., 2017; King et al., 2018; Monteiro et al., 2019b), and conform with evidence for higher thermal resilience at *L. digitata*'s warm distribution limit (King et al., 2019). Differentiation among Helgoland and Spitsbergen *L. digitata* was recently also shown by Martins et al. (2020), who experimented on a mixture of five gametophyte lineages, including the ones used in **Publication III**. There, Arctic gametophytes grew significantly slower than those from Helgoland at temperatures ranging from 15–25°C, and recruitment of sporophytes at 5 and 15°C was comparably reduced following a treatment >20°C for eight days (Martins et al., 2020). An increased performance of Arctic material at cold temperature became evident in the higher gametophyte fertility at 5°C following an eight-day treatment of 15°C (Martins et al., 2020). An increase in cold tolerance and reproduction at cold temperature are among the first steps towards adaptation to a cold climate, followed by reduced resilience to high temperature (Wiencke et al., 1994). These patterns are visible in the slight differentiation among the contrasting warm and cold range edge populations of *L. digitata* investigated here. Müller et al. (2008) were the first to describe differentiation among Arctic and cold-temperate *L. digitata*, as meiospore germination of Spitsbergen material was inhibited at 18°C, while ~90% of Helgoland meiospores germinated successfully. However, it cannot be ruled out that the Arctic *L. digitata* described by Müller et al. (2008) was actually *Hedophyllum nigripes*. *H. nigripes* co-occurs alongside *L. digitata* in the Arctic and is morphologically very similar (Dankworth et al., 2020), but presents a 4–5°C colder thermal profile (Franke, 2019). Olischläger and Wiencke (2013) reported meiospore release of Spitsbergen *L. digitata* sporophytes not before July, whereas Müller et al. (2008) collected meiospores in May, which indicates a potential confusion of the species. Therefore, sporophytes used in **Publication I** were confirmed as *L. digitata* by means of microsatellite amplification in addition to morphology, and material used in **Publication III** had been verified by DNA barcoding of sporophytes (Dankworth et al., 2020).

Answering research question I (Chapter 1.5), I provide evidence that populations of *L. digitata* have differentiated in their heat resilience along the species' Northeast Atlantic latitudinal distribution (Figure 6.1-I). As hypothesized, the population of Spitsbergen at the species' leading edge was less heat tolerant than the warm range-edge populations of Helgoland and Quiberon. Contrary to my hypothesis, genetic diversity and gene flow among populations were not evidently related to heat resilience, but the hierarchical genetic structure separating a northern and a southern genetic clade may have facilitated differentiation across glacial cycles. Although phenotypic differentiation corresponded to the prevailing thermal environment, differentiation was weak among the presumed ecotypes and was confined within the known thermal limits (Bolton and Lüning, 1982). Whether differentiation occurred also in response to cold temperature remains to be investigated.

6.1.2 Genetic variation for plasticity

In **Publication II**, I identified significant differences in phenotypic plasticity of growth and biochemical traits among five inbred genetic lines of *L. digitata* sporophytes within the population of Helgoland. Reaction norm slopes over the experimental temperatures of 5 and 15°C differed in height and direction (simplified in **Figure 6.1-II**), indicating genetic variation for plasticity which may allow kelp reaction norms to evolve during climate change (Newman, 1994). Genetic variation for plasticity was significant mostly in the group of sporophytes reared at 5°C, but due to the significant size differences among sporophytes reared at 5 and 15°C in **Publication II**, I cannot attribute this effect unambiguously to temperature.

Climate change increases the variability of environments (Thornton et al., 2014; Oliver et al., 2018) and therefore poses new demands for trait plasticity. High plasticity of a trait allows a genotype to respond to environmental gradients with a range of phenotypes, which may drive acclimative responses. In contrast, stability of e.g. reproductive traits may be favourable to ensure a functional phenotype which maintains fitness over environmental gradients. In the latter case, acclimation ensures a constant phenotype (i.e. phenotypic buffering; Reusch, 2014). Therefore differential trait expression may be adaptive or maladaptive depending on the trait and the variability of the environment, ultimately making trait plasticity itself a trait on which natural selection can act (Newman, 1994; Reed et al., 2011). Additionally, developmental temperature acclimation may modulate trait plasticity. This has recently been shown in the reduced morphological plasticity of *Macrocystis pyrifera* grown at sublethal temperature of 20°C (Supratya et al., 2020) and the reduced temperature sensitivity of photosynthesis and respiration of *Ecklonia radiata* when grown at a warm location (Staehr and Wernberg, 2009). This indicates that ocean warming may inhibit the expression of phenotypic plasticity in kelps, which decreases directional selection pressure and thereby lowers the adaptive capacity of populations. Genetic diversity was recently correlated to high phenotypic variation and resilience of kelp populations (Wernberg et al., 2018). Interestingly, I identified significant variation for plasticity in the *L. digitata* population of Helgoland, which contained comparably low genetic diversity (**Publication I**; King et al., 2020a; Neiva et al., 2020), but comparable studies to put these results into perspective are yet lacking for other populations.

These results confirm my hypothesis under research question II (Chapter 1.5) that thermal plasticity differs among *L. digitata* genotypes (Figure 6.1-II). Presumably similar genotypes, which were sampled only several metres apart within the same population, differed significantly in their expression of trait plasticity. Temperature and/or sporophyte age may further modulate trait plasticity, with indications for a stimulating effect of cold temperature on the range of plastic responses. These results indicate a potential of *L. digitata* populations to adapt to a range of sub- and supraoptimal temperatures if natural selection acts on genotypes differing in phenotypic plasticity.

6.1.3 Ontogenetic temperature history modulates thermal plasticity

Publication II revealed the importance of environmental history in shaping thermal plasticity of juvenile kelp sporophytes (simplified in **Figure 6.1-III**; **parental / carry-over effect**). Gametogenesis and recruitment at 5°C (blue dot in **Figure 6.1-III**), as opposed to 15°C (red dot in **Figure 6.1-III**), promoted higher growth of 3–4 month old offspring sporophytes at both experimental temperatures of 5 and 15°C, which is simplified as a higher blue reaction norm in **Figure 6.1-III**. This indicated either persistent parental effects across generations (from parental gametophyte F0 to offspring sporophyte F1) or carry-over effects across development within the sporophyte generation. Biochemical responses and chlorophyll fluorescence were highly plastic in response to the immediate temperature environment in the 12-day experiment and were not modulated by ontogenetic temperature history in clear patterns (**Publication II**).

The original experimental design of **Publication II** may be criticized for the overlap among generational treatments of gametophytes and newly recruited sporophytes. Newly recruited, few-celled sporophytes were exposed to the parental gametogenesis environment potentially for several days before being transferred to the sporophyte rearing treatment. As such a short environmental influence during the earliest developmental stages may theoretically affect phenotypic plasticity (Palmer et al., 2012; Donelson et al., 2018), I was unable to tease apart temperature effects across generations and within-generational effects. Building upon the results of **Publication II**, I therefore designed a study to explicitly investigate the hypothesis that kelps express cross-generational plasticity. This study was conducted in the frame of a Master's thesis (Gauci, 2020), which I co-supervised and which is now in preparation for publication. In the adapted experimental design, we circumvented the issue of overlapping generational treatments by testing thermal plasticity of offspring sporophytes obtained from parental gametophyte isolate strains which had been cultivated at 5 and 15°C for over three years. Gametogenesis and fertilization of both temperature cohorts were induced at a common temperature of 10°C. Subsequently, growth of microscopic juvenile sporophytes was assessed over 14 days in a temperature gradient ranging from 0 to 20°C (**Figure 6.2**). Thereby, we investigated if temperature treatments of the parental germ line would modulate thermal plasticity of recruited sporophyte offspring (i.e. cross-generational plasticity; Byrne et al., 2020). In a central result from his thesis, Gauci (2020) identified a beneficial effect of cold temperature applied during vegetative gametophyte growth on thermal resilience of microscopic sporophyte offspring at extreme temperatures. Microscopic sporophytes recruited from gametophytes grown at 5°C (pre-treatment 5°C; **Figure 6.2A**) significantly increased growth over time at 0°C, whereas sporophytes recruited from the gametophyte 15°C pre-treatment grew at an unchanged rate over time at 0°C (pre-treatment 15°C; **Figure 6.2B**). At 20°C, growth significantly decreased over time in sporophytes from both pre-treatments. However, sporophytes from the 15°C gametophyte pre-treatment completely ceased growth in the second week, while slow growth was

retained by sporophytes from the 5°C gametophyte pre-treatment. Mean sporophyte growth over two weeks at experimental temperatures of 0 and 20°C was therefore significantly higher in pre-treatment 5°C than in pre-treatment 15°C (Gauci, 2020), while growth did not differ significantly among pre-treatments at 5–15°C. These results therefore provide evidence for cross-generational plasticity in *L. digitata* (Gauci, 2020). In **Figure 6.1-III**, **cross-generational plasticity** is simplified as a change in reaction norm shape only at the thermal extremes. Following a gametophyte pre-treatment of 5°C (blue dot), growth of microscopic sporophytes was higher at the extreme temperatures of 0 and 20°C (blue lines) than in sporophytes from the pre-treatment of 15°C (red dot, red lines). Growth did not significantly differ among pre-treatments at temperatures from 5 to 15°C (grey line). This complies with the benefits of gametogenesis and recruitment at cold temperature described in **Publication II**.

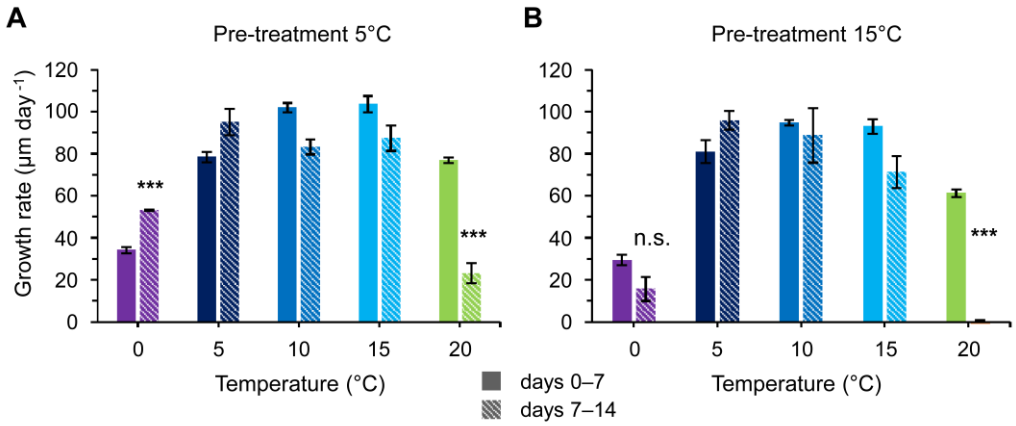

Figure 6.2 Length growth rates of microscopic *Laminaria digitata* sporophytes over two weeks in a temperature gradient (mean ± SE, n = 5). Sporophytes were obtained from gametophytes pre-cultivated for three years at 5°C **(A)** and 15°C **(B)**. Full bars represent growth during the first week of temperature treatment, striped bars represent growth during the second week of temperature treatment. Three-way repeated measures ANOVA returned a significant interaction of pre-treatment x temperature x time ($F(4,12) = 3.85$; $p = 0.031$). Significance of Tukey's pairwise comparisons among time points for 0 and 20°C is indicated in the Figures (***, $p < 0.001$; n.s., not significant). Figure adapted from Gauci (2020) and used with permission.

Answering research question II (Chapter 1.5), I provide evidence that ontogenetic temperature history across life cycle stages may alter thermal plasticity of juvenile offspring sporophytes in carry-over and/or cross-generational effects (Figure 6.1-III). Contrary to my initial hypothesis, performance of juvenile sporophytes did not increase at matching temperatures across ontogeny. Instead, a warm influence on microscopic *L. digitata* life cycle stages was correlated with reduced sporophyte growth across temperatures and with reduced resilience at extreme temperatures. This implies that cold seasons may be necessary for the persistence of *L. digitata* populations especially at the warm range edge. Despite the small magnitude of these effects in comparison to the range of plastic acclimation responses to the immediate thermal environment in the experiments, this raises the question if warming winters will reduce productivity and resilience of kelp populations in the long term. However, it remains unclear as yet if these effects persist also in older sporophytes.

6.1.4 Outbreeding may increase population resilience

Publication III demonstrates that outbreeding among populations may mitigate effects of genetic drift in small *L. digitata* populations and may lead to subtle heterosis effects (simplified in **Figure 6.1-IV**). Sporophytes of the Spitsbergen selfing showed an unexpectedly low heat tolerance as 19–20.5°C was lethal for macroscopic sporophytes, and the control temperature of 10°C elicited increased gene regulation compared to the Helgoland selfing. This was presumably an effect of inbreeding within the lineage rather than of local (mal)adaptation of the population, because thermal limits appeared uniform among individuals from the entire distribution range in **Publication I**. Resilience of Spitsbergen *L. digitata* at up to 22°C was recently also confirmed with laboratory-reared juvenile sporophytes, which were produced by crossing four gametophyte lineages in a Master's thesis (Franke, 2019). Still, the bleaching of one meristematic disc from Spitsbergen at 23°C during the heat stress experiment in **Publication I** indicates potential differences in heat resilience also among adult sporophyte individuals. In contrast, microscopic sporophytes of the Helgoland selfing had an upper survival temperature of 22°C over 14 days and macroscopic sporophytes persisted at 19 and 20.5°C over 17 days in **Publication III**. The differences in gene regulation at 10°C indicated genetically fixed differences among the inbred lineages of Helgoland and Spitsbergen. The reciprocal crosses showed heat resilience intermediate to their inbred parental lineages at 21 and 22°C, but percentages of unbleached microscopic sporophytes in the crosses were higher than the average value taken over both the Helgoland and Spitsbergen selfings (mid-parent heterosis; Hochholdinger and Hoecker, 2007). In experiment 2 on macroscopic sporophytes (**Publication III**), both reciprocal crosses responded similarly to the Helgoland selfing in growth and optimum quantum yield at sublethal heat of 19–20.5°C, whereas sporophytes from the Spitsbergen selfing bleached at these temperatures. Additionally, both crosses showed reduced differential gene expression at 20.5°C in comparison to the Helgoland selfing. This indicated lower metabolic costs for the crosses to persist during heat stress compared to the selfing (Clarke, 2003; Dekel and Alon, 2005). Transcriptomic responses were almost identical in a direct comparison among the reciprocal crosses, with only 11 differentially regulated genes (DEGs) at 20.5°C and only two DEGs at 10°C. This indicates that thermal traits were inherited mostly from both female and male gametophyte parents. Annotated differentially expressed genes indicated that cellular stress responses were reduced in the reciprocal crosses compared to the selfings. Potentially, increased heterozygosity may have benefitted the reciprocal crosses either by preventing the expression of deleterious recessive alleles or due to a general beneficial effect of heterozygosity across loci (Charlesworth and Charlesworth, 1987; Schierup and Christiansen, 1996).

The low dispersal capacity of kelps potentially prevents outbreeding across distant populations in the wild (Coyer et al., 1997; Billot et al., 2003). Rare events of dispersal (e.g. by drifting fronds; Schiel, 2004; Macaya et al., 2016) may already be sufficient to maintain gene flow among nearby

populations, preventing inbreeding depression and maintaining genetic homogeneity (Charlesworth and Charlesworth, 1987; Valero et al., 2011). However, the genetic structuring identified in **Publication I** refutes the hypothesis of natural outbreeding among *L. digitata* populations at a large geographical scale. The subtle effects of mid-parent heterosis demonstrated by the reciprocal crosses in **Publication III** were weak in comparison to the best-parent heterosis described for the interspecific crosses among the kelps *L. digitata* and *L. pallida*, which tolerated 2–3°C higher temperatures than either intraspecific selfing (Martins et al., 2019). Interspecific breeding of kelps is an established method to produce highly productive and resilient cultivars in mariculture (e.g. Li et al., 2007; Zhang et al., 2011). However, also intraspecific outbreeding among *Macrocystis pyrifera* lineages has been shown to increase fertility (Raimondi et al., 2004) and growth (Westermeier et al., 2010) compared to inbred cultivars which adds to the evidence for intraspecific heterosis in kelps. Potential detrimental effects indicating outbreeding depression were not obvious in performance of the reciprocal crosses among the contrasting *L. digitata* populations in **Publication III**. This suggests that genetic divergence among *L. digitata* populations is low (Schierup and Christiansen, 1996), despite the strong spatial structuring and thermal gradient along the species' latitudinal distribution (**Publication I**). Alternatively, outbreeding depression may manifest only in older sporophytes especially with respect to reproduction (Schierup and Christiansen, 1996; McKenzie and Bellgrove, 2006). Thus, it remains to be investigated whether outbreeding among *L. digitata* populations produces viable and fit offspring. In addition, genetic variation for plasticity (**Publication II**) may result in significant differences among cultivars regardless of whether they were in- or outbred (Camus et al., 2018). Therefore, careful assessments of cultivar productivity and fitness are necessary if outbreeding is considered for mariculture and/or restoration of natural kelp forests threatened by extinction.

Answering research question III (Chapter 1.5), I provide evidence that cross-breeding among differentiated lineages of *L. digitata* produced viable sporophytes which inherited thermal traits from both female and male parent gametophytes, and which expressed subtle heterosis (Figure 6.1-IV). As hypothesized, inbred Spitsbergen sporophytes were more sensitive to high temperature than inbred Helgoland sporophytes. However, this was likely not an expression of local adaptation, but potentially of inbreeding depression. Cross-breeding the Spitsbergen lineage with either female or male gametophyte parent from the more resilient Helgoland lineage produced viable, heat-tolerant sporophytes, which confirms my second hypothesis. Subtle heterosis was indicated for the reciprocal crosses, in that phenotypic heat responses were similar to the Helgoland selfing, while gene expression (e.g. of cellular stress response genes) was reduced in the reciprocal crosses at sublethal temperature.

6.1.5 Conceptual synopsis of thermal trait variability in a kelp

Thermal growth curves of *L. digitata* and other seaweeds (e.g. Fortes and Lüning, 1980; Bolton and Lüning, 1982; tom Dieck, 1992), on which I based the conceptual diagrams in this dissertation (**Figure 1.5**–**Figure 1.8**; **Figure 6.1**), roughly follow the shape of thermal performance curves described for ectothermic marine animals (Pörtner and Farrell, 2008; Pörtner, 2010). Over a temperature gradient, these curves often show a steady performance increase towards an optimum temperature, beyond which performance more steeply decreases (Fortes and Lüning, 1980; tom Dieck, 1992; Pörtner and Farrell, 2008). In ectothermic animals such as fish, thermal performance curves were described as integrating interactive temperature effects on the oxygen supply to tissue and on the kinetic speed of metabolic processes (concept of oxygen- and capacity-limited thermal tolerance; Pörtner, 2010). Optimum oxygenation of tissue occurs in a temperature range delimited by "pejus" (i.e. "turning worse") temperatures (Pörtner and Farrell, 2008; Pörtner, 2010). The kinetic stimulation of metabolic processes by warming, however, sets the integrative aerobic performance optimum of the animal close to the upper pejus temperature, resulting in a slightly negatively skewed bell curve (Pörtner, 2010). In photoautotrophic seaweeds, tissue oxygenation may not be the right process to explain thermal performance. Rather, the integrative trait of the thermal growth optimum may for seaweeds be shaped by the thermal optimum of the central process of photosynthesis, which is often higher than the optimum growth temperature (Eggert and Wiencke, 2000; Graiff et al., 2015; Fernández et al., 2020). Beyond optimum growth temperatures, performance is reduced by the metabolic investment in maintenance and repair mechanisms, and disruption of enzyme function and membrane structure (disruptive stress *sensu* Davison and Pearson, 1996; see also review by Eggert, 2012).

Considering the novel insights provided in this dissertation, it becomes clear that central thermal response mechanisms are not static within a species, but that variability of thermal traits in kelp is modulated by an interactive framework of genetic, non-genetic and environmental factors from scales of populations to individuals (**Figure 6.1**). On a large geographical scale, natural selection may drive genetic and phenotypic differentiation of populations (Kawecki and Ebert, 2004; **Figure 1.5**; **Figure 6.1-I**). Phenotypic differentiation at the ecological range edges (**Publication I**) is a pattern observed in several seaweeds (Gerard and Du Bois, 1988; Jueterbock et al., 2014; Saada et al., 2016; Mota et al., 2018) and is thought to be related to habitat isolation and unique genetic characteristics of range-edge populations (Kawecki and Ebert, 2004; Hampe and Petit, 2005; Sanford and Kelly, 2011). Local adaptation depends on interactions of genetic and environmental parameters (Kawecki and Ebert, 2004) which may be unique for every population of a species. One of the genetic mechanisms which facilitate local adaptation by shaping trait variability within a population is genetic variation for plasticity (Newman, 1994; **Figure 1.6**; **Figure 6.1-II**). If genotypes differ in their expression of plastic responses (**Publication II)**, directional selection on genetically and

phenotypically diverse populations may allow reaction norms to evolve (Via and Lande, 1985; Newman, 1994), potentially also in kelp populations (Coleman and Wernberg, 2020; Coleman et al., 2020a). However, this dissertation also provides evidence that increasing temperature during ontogeny may reduce the expression of thermal response plasticity (**Publication II**; **Figure 6.1-II**), growth (**Publication II**; **Figure 6.1-III**) and thermal resilience of juvenile kelp sporophytes (Gauci, 2020; **Chapter 6.1.3**; **Figure 6.1-III**) in non-genetic carry-over and/or cross-generational effects (**Figure 1.7**). These mechanisms are often investigated for their potential to mitigate effects of climate change (reviews by Donelson et al., 2018; Fox et al., 2019), but evidence is accumulating that they may not be a panacea in the face of climate change (Byrne et al., 2020). For kelp, I show evidence that carry-over and cross-generational effects may even drive detrimental responses during ocean warming. In contrast, outbreeding among differentiated populations may provide short-term rescue to genetically impoverished or maladapted populations (**Publication III**; **Figure 1.8**; **Figure 6.1-IV**). However, gene flow across large spatial scales is unlikely to occur naturally among distant populations of kelp and other macrophytes (Norton, 1992; Billot et al., 2003; King et al., 2018). While the other concepts presented here provide naturally occurring means of thermal trait variability, outbreeding among distant populations may provide an artificial means of increasing resilience of kelp populations to ocean warming.

For *Laminaria digitata*, the manifestation of these interactive levels of trait plasticity can be summarized as follows:

I. *L. digitata* populations differed slightly in their physiological responses to heat, indicating the existence of ecotypes (**Publication I**, **Chapter 6.1.1**, **Figure 6.1-I**). However, differentiation was weak and did not expand the thermal tolerance range beyond the known upper thermal limit. Especially the northernmost population showed reduced function during heat exposure, which may be due to a lack of selection pressure for heat tolerance in the Arctic. Whether ecotypic differentiation has taken place with respect to cold temperature is still unknown.

II. The expression of thermal plasticity differed among genotypes (**Publication II**, **Chapter 6.1.2**, **Figure 6.1-II**). This was evident even among individuals sampled from the same population which were presumably not strongly differentiated genotypes. Differences in plasticity may be weak, but cold temperature and/or sporophyte age may stimulate the expression of plastic responses. These results imply that, despite the mostly uniform upper thermal limit, differentiation in trait responses to a range of sub- and supraoptimal temperatures is possible among individuals and populations of *L. digitata*.

III. Carry-over effects and/or cross-generational plasticity may modulate thermal plasticity of *L. digitata* (**Publication II**, **Chapter 6.1.3**, **Figure 6.1-III**). Effects in juvenile sporophytes were only beneficial following cold temperature treatments of reproduction and ontogeny

compared to warm treatments, indicating the importance of cold seasons for population persistence. These effects were of smaller magnitude than rapid acclimation responses to the immediate thermal environment in the experiments, and it remains unclear whether they persist also in older sporophytes.

IV. Intra-specific outbreeding among differentiated lineages produced viable sporophytes with subtle expression of heterosis (**Publication III**, **Chapter 6.1.4**, **Figure 6.1-IV**). Contributions from both female and male parent gametophytes shaped the thermal response of offspring sporophytes. Whereas inbreeding may reduce the thermal resilience and performance of *L. digitata* sporophytes, increased heterozygosity in the crossed sporophytes potentially increased their performance during heat exposure. However, the known thermal growth limits were not surpassed by the crossed lineages.

In summary, *L. digitata* is a species with a clear cold-temperate to Arctic adaptation, which expresses thermal trait variability on different levels, but is generally restricted by a nearly uniform upper thermal limit. Response variation among individuals within the thermal limits may be more important for the adaptive capacity of the species than the current differentiation of ecotypes at the extremes of the thermal tolerance range. The small magnitude of the above phenomena and the potential negative effects of warming winters indicate that these mechanisms may not allow the species to persist during climate change at its current warm range edge, which I will discuss in detail in the following chapter.

6.2 Thermal plasticity of *Laminaria digitata* in a changing climate

In this chapter, I will explore potential consequences and applications based on the above findings which contribute to the understanding of thermal plasticity in kelp and *Laminaria digitata* in particular. I discuss the role of trait variability and genetic differentiation in the light of ocean warming and marine heatwaves in particular, and I explore potential directions for research on and with kelp regarding fundamental research, applied mariculture and restoration efforts for threatened natural populations.

6.2.1 Phenology of *Laminaria digitata* under ocean warming

The differing thermal optima and limits for ontogenetic traits of gametophytes and sporophytes (**Figure 1.4**) imply that phenology of *L. digitata* is coupled to seasonal temperature variation, especially at the warm range margins. In northern to Arctic populations, where sea surface temperatures (SST) oscillate between non-inhibiting temperatures of 0 and 15°C (e.g. Barents Sea, Makarov et al., 1999; mid-northern Norway and Spitsbergen, **Publication I**), phenology is mainly

controlled by the seasonality of irradiance, photoperiod and nutrients (reviewed by Wiencke et al., 2009). Photoperiod synchronizes the endogenous growth rhythm in *L. digitata* (Schaffelke and Lüning, 1994; Makarov et al., 1999) which in turn affects the onset of sporogenesis during periods of low growth (Buchholz and Lüning, 1999). Temperature and nutrient availability may then modulate the efficiency of sporophyte fertility (**Figure 1.4**; Buchholz and Lüning, 1999; Bartsch et al., 2013). Long day lengths additionally enhance gametophyte and sporophyte fertility across temperature gradients (Hsiao and Druehl, 1971; Lüning, 1988; Martins et al., 2017), whereas both darkness and cold temperature inhibit gametogenesis in the polar winter (Lüning, 1980; Sjøtun and Schoschina, 2002).

At locations which reach high temperatures inhibiting reproductive traits in summer, such as Helgoland and Quiberon (**Publication I**; Bartsch et al., 2013; Oppliger et al., 2014), seasonal temperature variation has a larger effect in controlling *L. digitata*'s life cycle (Martins et al., 2017). Sporophytes release meiospores in summer and autumn, including periods when seawater temperatures are maximal (Bartsch et al., 2013). Released meiospores may suffer damage from interactive effects of high temperature and UV radiation in summer (Roleda et al., 2005; Roleda, 2009), whereas sporangia likely provide protection from UV radiation (Gruber et al., 2011). Once meiospores have settled and germinated, the high thermal resilience and warm growth optimum of gametophytes of up to 18°C (**Figure 1.4**; Lüning, 1980; Martins et al., 2017) ensures vegetative persistence at temperatures ≥ 18°C during summer, which may be lethal already for sporophytes impacted by multiple stressors especially during emersion (Bartsch et al., 2013). In the absence of *in situ* data on gametophyte development, gametogenesis is thought to subsequently occur in late autumn at temperatures ≤ 17°C or in early spring at lowest annual temperatures (Martins et al., 2017), but likely not at minimal irradiance during winter (Lüning, 1980). Juvenile recruited sporophytes may grow and develop from early spring at temperatures increasing again to their thermal growth optimum of 10–15°C (**Figure 1.4**). According to the results of **Publication II**, a seasonal pattern of recruitment and juvenile sporophyte growth at yearly minimum temperatures benefits growth, thermal resilience and the expression of trait plasticity of juvenile sporophytes.

Due to the coupling of temperature and ontogeny, mean warming of the Atlantic by 2–4°C until the end of the century in the maximum greenhouse gas emission scenario (RCP 8.5; Hoegh-Guldberg et al., 2014; IPCC, 2019) will potentially disturb the phenology of *L. digitata*. The earlier onset of the warm season along most global coastlines (Lima and Wethey, 2012) has already led to shifts in phenology of many marine taxa, e.g. earlier phytoplankton blooms (reviewed by Poloczanska et al., 2013). Because the onset of sporogenesis is likely controlled indirectly by the photoperiod which synchronizes the circannual growth rhythm (see above; Buchholz and Lüning, 1999; Bartsch et al., 2013), an earlier onset of the warm season will likely not affect the seasonal timing of sporophyte

fertility. For instance, during two years differing in spring sea surface temperature by few degrees, *L. digitata* sporophytes on Helgoland initiated sporogenesis at the end of April (Bartsch et al., 2013).

More importantly, the shortening of the cold season combined with an increase of summer periods in which sporogenesis and gametogenesis are inhibited potentially reduces the fertile window of *L. digitata* and may initiate a negative feedback loop. Hypothetically, reduced meiospore production and germination at high temperature (Bartsch et al., 2013) may result in a lower number of gametophytes to recruit juvenile sporophytes. In consequence, incremental reductions in recruitment over generations may reduce population size and genetic diversity of the population (Vucetich and Waite, 2003). Therefore, population size, diversity and resilience (Wernberg et al., 2018) may be reduced even in the absence of extreme events such as marine heatwaves. This might especially occur towards the warm range edge, where offspring sporophyte performance presumably depends more on the reproduction during cold seasons than in more northern populations (**Publication II**; **Chapter 6.1.3**). *L. digitata* may already exhibit reproductive maladaptations at the species' southern European range limit, as sporophytes produce a low amount of spores, of which a relatively high fraction are diploid instead of haploid meiospores (Oppliger et al., 2014). This was attributed by the authors to the warm thermal regime at the species' trailing edge. Subtle differentiations like this may therefore manifest during ocean warming also at more northern populations for *L. digitata*. For other kelps, especially those that produce spores primarily in winter (e.g. *L. hyperborea*, *S. latissima*; Fredriksen et al., 1995; Bartsch et al., 2008; Sogn Andersen et al., 2011), effects of ocean warming on the life cycle may manifest in different ways and warrant further investigation.

6.2.2 Marine heatwaves – kelp responses and consequences

Following an extreme marine heatwave in Western Australia in 2011, which drove a well-documented regime shift from marine forest to turf algae (Wernberg et al., 2016), the responses of kelp species and marine forests to heat and marine heatwaves in particular have received increased attention (Holbrook et al., 2020; see e.g. Wilson et al., 2015; Arafeh-Dalmau et al., 2019; Nepper-Davidsen et al., 2019; Gurgel et al., 2020). Heat stress responses of kelp sporophytes are meanwhile well-described for integrative physiological parameters. At sublethal temperature, kelps reduce growth, the integrative parameter over all metabolic processes (**Publication I**; tom Dieck, 1992; Wilson et al., 2015), while energy is channelled into stress responses (López-Maury et al., 2008). Carbon assimilation is reduced (Pessarrodona et al., 2018; Nepper-Davidsen et al., 2019) while at the same time, distal loss of blade tissue increases due to tissue damage and reduced mechanical resilience (Simonson et al., 2015; Nepper-Davidsen et al., 2019). The decrease of carbon assimilation at increasing temperatures is related to dynamic photoinhibition, which is visible as a reversible

decrease of optimum quantum yield F_v/F_m (**Publication I**; Nepper-Davidsen et al., 2019; Hereward et al., 2020) before damage occurs to the photosystem in chronic photoinhibition (Hanelt, 1998). To prevent an accumulation of reactive oxygen species (ROS) which may over-reduce and damage the photosynthetic electron transport chain (Suzuki and Mittler, 2006), kelps may increase concentrations of carotenoid accessory pigments, especially xanthophylls, which dissipate excess energy from the photosystem (nonphotochemical quenching; **Publication I**; Li et al., 2019; Nepper-Davidsen et al., 2019). Additionally, an accumulation of flavonoid and polyphenolic defense compounds increases the antioxidant capacity while enduring periods of critical heat stress (Hargrave et al., 2017). Nitrate availability has been shown to increase heat resilience of *Macrocystis pyrifera*, presumably due to the availability of nitrogen for increased production of heat shock proteins and modification of cell membranes (Fernández et al., 2020). However, cellular nitrogen contents do not show consistent responses to high temperature across kelp populations and species (**Publication I**; Roleda and Hurd, 2019).

The first study on gene expression underlying phenotypic stress responses of *L. digitata* was published 15 years ago (Roeder et al., 2005) and allowed to identify changes to metabolic pathways before they translate into phenotypic responses (Heinrich et al., 2015). With the advent of affordable next-generation sequencing, research of whole transcriptomes has gained increased attention in research on kelp responses to abiotic stress (e.g. Heinrich et al., 2012; Iñiguez et al., 2017; Monteiro et al., 2019b). Several molecular mechanisms in response to stress are evolutionarily conserved and are collected under the term of cellular stress response (CSR; Kültz, 2005). For *L. digitata*, I identified numerous differentially regulated genes from the CSR categories of redox regulation, molecular chaperones and lipid metabolism during heat stress, in addition to regulation of pigment and carbohydrate metabolism (**Publication III**). These are in accordance with the phenotypic changes observed in response to heat. Modifications of membrane and cell wall rigidity by altering carbohydrate and lipid composition (**Publication III**) may drive changes in thallus morphology and reduction of mechanical resilience while growth is reduced (Simonson et al., 2015; Supratya et al., 2020). Regulation of pigment synthesis and photosystem components corroborates the maintenance of photosynthesis during heat stress which was visible in the stability of optimum quantum yield F_v/F_m (**Publication III**). Meanwhile, the regulation of ROS-scavenging and ROS-producing proteins implies cellular oxidative stress and signalling in response to heat (Bischof and Rautenberger, 2012). The uncontrolled production of ROS occurs through disturbances of the homeostasis of electron transport chains in photosynthesis, photorespiration, or mitochondrial respiration (Bhattacharjee, 2005), and is met by production of ROS-scavenging antioxidants which prevent destructive oxidation of lipids, proteins and nucleic acids (Bischof and Rautenberger, 2012). Finally, the induction of heat shock protein genes (**Publication III**) indicates that protein function is impaired at high temperature

and has to be supported by chaperone proteins, which ensure correct protein folding, translocation and controlled degradation (Wang et al., 2004).

While these responses are expressed during tolerance of heat stress, the capacity to return to the original metabolic state is important for the long-term persistence of populations. *L. digitata* suffered persistent damage from five days at 23°C, as growth did not recover during the following week at 15°C (**Publication I**). In a similar experiment, *Saccharina latissima* persisted during a simulated heatwave at 18 and 21°C for 23–27 days, but did not recover to full potential of growth and photosynthesis during 2 weeks of post-cultivation at 15°C. In the seagrass *Zostera marina*, transcriptomic stress profiles during an experimental three-week heatwave of 26°C were similar among two populations from Denmark and Italy, but only the Italian population returned to control values of gene expression following a temperature decrease to 19°C (Franssen et al., 2011), indicating a difference among ecotypes in the potential to recover. Therefore, even if populations persist during a marine heatwave, lingering stress and/or damage may become evident later in time. Currently, marine heatwaves already reach temperatures of 20°C in South England (Joint and Smale, 2017; Burdett et al., 2019). In combination with stress during emersion at lowest tide, especially air temperature and desiccation (Hereward et al., 2020), these temperatures may already drive die-off events of sporophyte populations such as on Helgoland in 2003 (Bartsch et al., 2013). Therefore, the predicted increase in frequency and duration of marine heatwaves (Oliver et al., 2018) poses an additional threat to *L. digitata* at the warm range edges.

The resilience of Australian *Ecklonia raditata* populations to disturbances such as marine heatwaves correlated with neutral genetic diversity within populations, presumably due to increased physiological versatility among individuals (Wernberg et al., 2018). **Publication II** provides first evidence for variation in thermal plasticity among *L. digitata* individuals from Helgoland (**Figure 6.1-II**), but analogous data from other populations are necessary to evaluate the relationship of genetic diversity and response variation in a comprehensive context for multiple populations. Marine heatwaves can act as strong disturbance events and may pose bottlenecks for genetic diversity by random selection (Gurgel et al., 2020) which reduces diversity and resilience of populations. Whereas neutral genetic diversity may be an indicator of trait variation, it does not allow direct derivation of local adaptation, as microsatellite markers are by definition not under selection pressure (Ellegren, 2004; Vieira et al., 2016). Populations with reduced neutral genetic diversity characteristics may still be performing well under thermal stress if the population has adapted to the local environment. For instance, sporophytes from the population of Helgoland presented better heat resilience than samples from other, more genetically diverse populations in **Publication I** (**Figure 6.1-I**). The higher heat resilience of South English *L. digitata* populations compared to more northern, Scottish populations (King et al., 2019) also coincides with reduced genetic diversity in the southern populations (King et al., 2020a). This was interpreted by King et al. (2020a) as a reduction

of historically higher diversity due to the challenging habitat and reduced connectivity at the species' trailing edge (Eckert et al., 2008), which may also be applicable to the population of Helgoland (**Publication I**). Marine heatwaves may not only pose random selective events, but they have recently been hypothesized as also driving directional selection for thermal traits (Coleman and Wernberg, 2020; Coleman et al., 2020a), and may therefore be one of the factors driving ecotypic differentiation for thermal tolerance at the range edges.

Current research on marine heatwaves often focuses on extreme summer temperatures, but **Publication II** demonstrates that seasonal cold temperatures may also be important for the performance and persistence of *L. digitata* populations (**Figure 6.1-III**). Indeed, marine heatwaves during winter can drive competitive disadvantages of native seaweeds competing with warm-adapted invasive species, as was shown for the invasive *Sargassum muticum* at the Welsh coast (Atkinson et al., 2020). This was in part due to reduced thermal resilience of the native *Fucus serratus* and *Chondrus cripsus* in winter (Atkinson et al., 2020). Thermal tolerance of recently grown kelp tissue in late winter to early spring was reduced compared to older tissue in summer for *L. digitata* (by 2°C) and *L. hyperborea* (by 5°C), among other seaweeds on Helgoland (Lüning, 1984). Therefore, warming winters may, for instance, support the northward propagation of the warm-temperate kelp *L. ochroleuca* in the Southern United Kingdom (Smale et al., 2015; Hargrave et al., 2017). **It is becoming evident that ocean warming does not only pose a stressor in summer at highest temperatures, but affects phenology, plasticity and species interactions year-round.**

6.2.3 Adaptive capacity of *Laminaria digitata* during ocean warming

The abovementioned effects contribute to the poleward range shifts of seaweeds predicted already 30 years ago (Breeman, 1990). In recent niche models, habitat suitability of *L. digitata* was best described based on sea surface temperature (SST), salinity and bathymetry (Raybaud et al., 2013; Assis et al., 2018). Whereas Raybaud et al. (2013) only considered maximum SST in their best-fitting model, Assis et al. (2018) also took into account maximum winter temperature, and predicted a loss of suitable habitat area of up to 16% until the end of the century. While *L. digitata* biomass has in part been receding near its southern range limit (e.g. Normandy, France; Cosson, 1999), *L. digitata* biomass has significantly increased at the leading edge in the Arctic Kongsfjorden, Spitsbergen, between 1996 and 2013 (Bartsch et al., 2016). With respect to predicted warming, current range central populations might reduce their primary productivity in the future as supraoptimal thermal conditions become more likely (King et al., 2020b). Bearing in mind these changes and predictions, the question arises how well *L. digitata* as a species is equipped to persist and adapt to a changing climate.

I identified response variation on the scale of populations and individuals for *Laminaria digitata*. Despite evidence for differentiation of thermal traits among populations, differentiation was of rather minor extent and did not affect the upper thermal limit of wild *L. digitata* sporophytes (**Publication I**; **Figure 6.1-I**). Additionally, evidence for local adaptation identified under laboratory settings does not equal high productivity of the respective natural population, as is exemplified by the lower *in situ* primary production of the warm-adapted South English *L. digitata* ecotype compared to range central populations (King et al., 2019, 2020b). Therefore, potential local adaptation may not be sufficient to buffer loss of sporophytes at southern *L. digitata* populations during increasingly warm summers. However, evidence for heat resilience of reproductive traits of gametophytes (Martins et al., 2020) indicates that gametophytes may, at least for some generations, replenish the sporophyte populations after die-off events in summer, such as on Helgoland between 2003 and 2004 (Bartsch et al., 2013). Potentially more important for the adaptive capacity of the species than present local adaptation is the presumed high genetic variation for plasticity among genotypes (**Publication II**; **Figure 6.1-II**). The fact that thermal reaction norms of several traits differed among individual genetic lines implies that thermal traits have the potential to evolve within the thermal limits of the species (Newman, 1994; Reed et al., 2011), and that strong selection on genetically (and phenotypically) diverse populations may drive adaptive processes (Coleman and Wernberg, 2020; Coleman et al., 2020a). However, indications that increasing temperature during ontogeny may reduce response plasticity and growth of juvenile sporophytes (**Publication II**; Staehr and Wernberg, 2009) imply that the same process of warming, which exerts a selective pressure on kelp sporophytes, might simultaneously decrease the strength of directional selection by reducing response plasticity in kelps.

While populations at the trailing edge in Brittany are at risk of extinction, *L. digitata* biomass is increasing in Arctic populations (Bartsch et al., 2016). In the Arctic, increasing temperature does not pose a stressor for *L. digitata*. However, the Arctic is warming rapidly by 3–4°C until the end of the century (Müller et al., 2009), which increases sedimentation and reduces seawater salinity due to permafrost erosion, glacial melting and freshwater runoff (Peterson et al., 2002; Filbee-Dexter et al., 2019). Increasing seawater turbidity drives a shift of Arctic *L. digtata* to shallower depths (Bartsch et al., 2016) while freshwater input may induce hyposaline stress (Li et al., 2019; Monteiro et al., 2019b). Still, models predict an overall increase of kelp forest extent and biomass in the Arctic due to reduced sea ice cover and warming (Assis et al., 2018; Filbee-Dexter et al., 2019). **The prospect of loss of warm ecotypes and dispersal of cold ecotypes of *L. digitata* implies a decrease in the adaptive capacity to warming of the entire species.** The strong spatial structuring among kelp populations (Billot et al., 2003) implies that the unique genetic diversity in glacial refugia populations at *L. digitata*'s trailing edge (Assis et al., 2018; Neiva et al., 2020) will likely not be "rescued" by connectivity and gene flow to more northern populations. Rare, long-distance gene flow events may hypothetically introduce new alleles to a population (Schiel, 2004) and might even produce more

resilient phenotypes (**Publication III**; **Figure 6.1-IV**), but the low connectivity among extant *L. digitata* populations suggests that this does not occur regularly for *L. digitata* (**Publication I**; King et al., 2020a; Neiva et al., 2020). The low degree of connectivity among *L. digitata* populations in Brittany is rather explained with gradual gene flow via stepping stone habitats (as hypothesized in **Publication I**).

Further aspects which niche models do not take into account are biotic interactions and competition among species which may be differentially affected by climate change. Marine forests are structured differently along the latitudinal gradient according to the thermal characteristics of key kelp species, e.g. higher abundance of *Alaria esculenta* and *Saccharina latissima* in cold-temperate Northern Europe and increasing abundance of *Laminaria ochroleuca* towards warm-temperate Southern Europe (Smale and Moore, 2017; Smale, 2020). *L. ochroleuca* is extending its range northward, which is documented well in South England, where it competes with *L. hyperborea* (Smale et al., 2015). The high abundance of gastropod grazers associated with *L. ochroleuca* is contrasted by a high amount of epiphytes growing on the stipes of *L. hyperborea* (Smale et al., 2015). Taking into account also the differential patterns in seasonal growth and carbon export (Pessarrodona et al., 2019), a replacement of *L. hyperborea* or *L. digitata* by *L. ochroleuca* may drive trophic shifts in kelp forests based on the different ecological function of the key species. However, *L. ochroleuca*'s low tolerance to desiccation and freezing might prevent a replacement of *L. digitata* in the infralittoral fringe (Hargrave et al., 2017; King et al., 2018). Following the experimental removal of *L. digitata* at sites in Brittany, France, the warm-temperate annual kelp *Saccorhiza polyschides* (Tilopteridales) recruited quickly and the ecosystem took between 18–24 months to revert to its initial state (Engelen et al., 2011), which additionally highlights the impact of single disturbance events on population persistence. Under Arctic summer warming scenarios at 9–10°C, co-cultivation of gametophytes and sporophytes indicated a competitive advantage of *A. esculenta* over *L. digitata* in development and growth under laboratory conditions (Zacher et al., 2019). In turn there is evidence that *L. digitata* might outcompete the cold-adapted *Hedophyllum nigripes* where they co-occur in the warming Arctic (Franke, 2019). Lastly, shifts throughout the food web may control abundance of kelps forests in multitrophic cascades. For instance, grazing by sea urchins can remove entire marine forests (Sivertsen, 2006), but they might recover if ocean warming facilitates an increase in abundance of urchin predators such as crabs (Christie et al., 2019b).

This amounts to the conclusion that the adaptive capacity to climate change of kelp species in general and *Laminaria digitata* in particular is a highly variable and complex framework which cannot be defined globally. Responses of *L. digitata* to ocean warming are shaped by interactive effects of the species' genetic diversity, local adaptation, population structure, local environmental conditions, strength and direction of selective forces, mating system, within-generational and cross-generational phenotypic plasticity, and biotic interactions with competitors and herbivores. Thermal trait

variability is thereby an important aspect of *L. digitata*'s response to ocean warming, but care should be taken when extrapolating evidence from laboratory experiments to conditions in the wild. **Yet, what has become increasingly obvious during my investigations is the consistent adaptation of *L. digitata* as a cold-temperate to Arctic species. *L. digitata* may persist at temperatures > 20°C (Publication I; Publication III), but it thrives at 5–15°C (Figure 1.4; Figure 6.2; Publication II) and neither local adaptation, within- or cross-generational plasticity nor heterosis were shown to improve the species' performance at high temperature beyond the limits that were described almost 40 years ago (Bolton and Lüning, 1982).** Within this century, mean Atlantic sea surface temperatures are predicted to increase by a range of 2–4°C (RCP 8.5; Hoegh-Guldberg et al., 2014; IPCC, 2019) while marine heatwaves increase in frequency and duration (Oliver et al., 2018). Taking into account the relatively weak differentiation among populations, and the potentially negative effects of warming winters on kelp sporophyte performance, **it is unlikely that the diverse range of thermal trait variability of *L. digitata* will prevent an extinction of the threatened populations at the species' warm range edge**. Still, the variability of trait responses to sub- and supraoptimal temperature indicates that *L. digitata* possesses the potential to adapt within the species' eurythermal performance range which may benefit range central and northern populations under ocean warming.

6.3 Perspectives and opportunities for kelp research

In this chapter I discuss perspectives for research on and with kelp which build on the concepts and hypotheses presented within the scope of this dissertation. The methodological development of fundamental research on kelps is not as advanced as for plant and animal research (Chan et al., 2006). Still, the increasing availability of genomic, transcriptomic and epigenetic resources for brown algae (e.g. **Publication III**; Cock et al., 2010; Heinrich et al., 2012; Monteiro et al., 2019a; Coelho et al., 2020) provides valuable tools to complement manipulative experiments and investigate the molecular basis of trait variation and plasticity in the future.

To further investigate local adaptation among kelp populations (**Publication I**), the first aim should be to verify that any phenotypic differences among populations are of genetic origin. To rule out potential cross-generational effects, common garden experiments should be conducted on subsequent offspring generations (F2+) of sporophytes and gametophytes which were reared in a common laboratory environment (King et al., 2018). Ideally, at least 20 gametophyte isolates are obtained each from range central and marginal populations to experiment on replicated genetic lines and relate genetic variation for plasticity (*sensu* **Publication II**) to neutral genetic diversity among populations. Further, experiments on key traits related to fitness should take into account their phenology in the wild. For instance, *L. digitata* sporophyte reproduction should be assessed in a warm temperature

gradient at long day length, and gametophyte fertility in a cold temperature gradient at short day length, to be relatable to summer and winter conditions along the latitudinal distribution range.

Additionally, further research among kelp selfings and crosses is necessary to understand effects and mechanisms involved in inbreeding and outbreeding (**Publication III**). To quantify effects of inbreeding depression and heterosis, optimum performance and thermal tolerance may be tested in physiological experiments comparing sporophytes of at least 20 lineages (see above) of selfings, within-population crosses and across-population crosses. In cooperation with mariculture, controlled *in situ* experiments on cultivation lines may allow to assess also the fertility of adult sporophytes to take into account that effects may manifest later in the life cycle (Raimondi et al., 2004).

A major objective in researching local adaptation and heterosis is to link genetic characteristics to phenotypic responses (Pardo-Diaz et al., 2015; Hoban et al., 2016; Yang et al., 2017; King et al., 2018). Instead of neutral microsatellite markers, which do not allow inferences of selective forces, adaptive markers which are under selection pressure may be linked to local adaptation (Coleman et al., 2020a). Laboratory experiments should therefore be complemented by molecular analyses, e.g. of genome-wide single nucleotide polymorphisms (SNPs) which may serve as neutral and adaptive markers (Heylar et al., 2011; Batista et al., 2016). They may therefore allow to identify quantitative trait loci (QTL) which are linked to adaptive traits (Liu et al., 2010; Price et al., 2018) and expression of heterosis (Yang et al., 2017).

Further research on cross-generational plasticity and carry-over effects (Publication II) should investigate their magnitude and significance in the wild. To investigate how kelp reproduction during ocean warming affects offspring sporophyte performance *in situ*, replicated genetic lines initiated from gametophyte isolates should be subjected to temperature pre-treatments simulating gametogenesis and recruitment (*sensu* **Publication II** and Gauci, 2020) under control and warming scenarios for autumn, winter and spring. The resulting offspring sporophytes with different temperature histories should then be transplanted to the field to quantify growth and fertility *in situ*, which will allow to estimate the significance of non-genetic effects on the performance of natural populations.

A range of non-genetic mechanisms may be underlying cross-generational plasticity in kelps. To strengthen the evidence for CGP, further experiments manipulating the sporophyte environment before sporogenesis should be conducted to confirm whether effects persist in next-generation sporophytes across meiosis, gametogenesis and fertilization. Offspring sporophytes may then be investigated for molecular mechanisms modulating their trait plasticity. For instance, evidence for DNA methylation was recently detected in kelp gametophytes and sporophytes (Qu et al., 2013; Fan et al., 2020) which can mediate response variation in the absence of genetic variation, as was recently shown in seagrass (Jueterbock et al., 2020). Analysis of histone modifications suggests that protein-

DNA interactions shape response variation in *E. siliculosus* (Bourdareau, 2018; Coelho et al., 2020). Additionally to these epigenetic mechanisms, parental effects may be mediated by somatic mechanisms such as provisioning of propagules (Lundgren and Sultan, 2005), which in the case of kelps may be through neutral lipids (Brzezinski et al., 1993; Steinhoff et al., 2011).

What cannot be considered in laboratory experiments is the obvious assumption that all of the above effects act interactively in the wild. Therefore, a comprehensive approach to investigate the integrative metabolic response of wild populations to the prevailing environment, including any potential effects of local adaptation, within- and cross-generational plasticity, would be to conduct a comparative transcriptomic study among hundreds of wild sporophytes sampled at the same time of year across the entire latitudinal distribution, e.g. during the warmest and/or coldest month. Such a comprehensive dataset will, provided funding and workforce, allow to identify key processes shaping performance and resilience of marine forests while also providing population genetic information based on SNPs in the transcripts (Jueterbock et al., 2016).

Insights from fundamental research may then be applied to kelp mariculture and restoration. Selective breeding programs are producing resilient and productive kelp cultivars for the Asian seaweed industry (Li et al., 2007, 2008; Zhang et al., 2011), but the genetic science behind seaweed breeding and domestication is still in an initial phase (Westermeier et al., 2010; Robinson et al., 2013; Camus et al., 2018). The identification of alleles for high yield growth characteristics (Liu et al., 2010; Xu et al., 2015) and genetic loci associated with heterosis (Yang et al., 2017) will allow more targeted breeding based on genetic and phenotypic traits. Pre-treatments on microscopic stages before cultivation in the field may then make use of within- and cross-generational effects to maximize crop yield. Additionally, these methods may be applied in efforts of "assisted adaptation" to restore and maintain threatened marine forests (Filbee-Dexter and Smajdor, 2019; Wood et al., 2019; Coleman et al., 2020b). As reviewed by Coleman et al. (2020b), population resilience may be reinforced by introducing genetic diversity ("genetic rescue"), introducing selected genotypes with desirable traits ("assisted gene flow"), or by making use of heterosis by cross-breeding ecotypes. Persistent beneficial effects of artificial outbreeding have already been shown over multiple generations of plants (Willi et al., 2007) and animals (Lindsay et al., 2020) in the wild. Whether these methods should be applied to natural kelp populations is highly debatable, as it is centred around creating value for humans rather than preservation of what we deem "natural", but which is lost in our industrialized world (Filbee-Dexter and Smajdor, 2019). Therefore, the application of these methods on wild marine forests is ultimately not a question of technique, but a question of ethics, which we have to carefully consider from now on.

6.4 Conclusion

Responsiveness to an environment is one of the key characteristics of life (Koshland Jr., 2002). If differences in these responses among individuals are heritable, natural selection may drive adaptation of populations and evolution of a species. This has been a central principle of biology for more than 150 years (Darwin, 1859). Still, current research is investigating the diverse mechanisms underlying such response variability, meanwhile often with the background of anthropogenic climate change and its impact on nature. Making a small contribution to this fundamental objective, the results presented in this dissertation provide evidence for thermal trait variability of the forest-forming kelp *Laminaria digitata* on different scales from populations to life cycle stages. On large spatial scales, *L. digitata* populations have differentiated slightly in their thermal resilience in accordance to the prevailing climate. Within populations, I provide evidence for genetic variation for phenotypic plasticity among individuals, which may allow directional selection and adaptation during ocean warming. Temperature also modifies the plasticity of traits across generations and individual ontogeny, potentially mitigating or amplifying effects of ocean warming. By artificially cross-breeding differentiated lineages within the species, new phenotypes may be created. Integrating these responses into a framework of seasonal temperature variation in the field and predictions of ocean warming showed that *L. digitata*, as a cold-temperate to Arctic species, is adapted well to the current conditions along its distributional range, but may not be equipped to respond to rapid climate change at its warm range edges. Discussion has been initiated whether to artificially maintain marine forests threatened by climate change, and some of the concepts investigated in this dissertation may be investigated further for such applications, namely genetic variation for plasticity, heterosis, and cross-generational plasticity. Which molecular processes are driving these effects is largely unclear as yet, but the haplo-diplontic life cycle of kelps provides a unique experimental system to further explore these mechanisms. The experimental designs, concepts and data discussed in this dissertation therefore contribute to the growing knowledge of trait variability and the adaptive capacity of kelps, and will hopefully inspire further research.

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