Research article

Kiara Franke*, Daniel Liesner, Svenja Heesch and Inka Bartsch

Looks can be deceiving: contrasting temperature characteristics of two morphologically similar kelp species co-occurring in the Arctic

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Abstract: Two morphologically similar digitate kelp species, *Laminaria digitata* and *Hedophyllum nigripes*, co-occur along a shallow sublittoral depth gradient in the Arctic but, in contrast to *L. digitata*, very few ecophysiological data exist for *H. nigripes*. We investigated growth, survival, photosynthetic characteristics and carbon:nitrogen ratios of juvenile sporophytes, and recruitment and survival of gametophytes in genetically verified Arctic isolates of both species along temperature gradients (0–25 °C) over 14 days. *Laminaria digitata* gametophytes survived 23–24 °C, while sporophytes survived 21–22 °C. *Hedophyllum nigripes* had lower temperature affinities. Gametophytes survived 19–21 °C, while sporophytes survived 18 °C. Male gametophytes were more heat-tolerant than female gametophytes in both species. The pronounced cold adaption of *H. nigripes* compared to *L. digitata* also became apparent in different sporophyte growth optima (*L. digitata*: 15 °C; *H. nigripes*: 10 °C) and gametogenesis optima (*L. digitata*: 5–15 °C; *H. nigripes*: 0–10 °C). Higher carbon:nitrogen ratios in *H. nigripes* suggest an adaptation to nutrient poor Arctic conditions. The overall temperature performance of *H. nigripes* possibly restricts the species to Arctic–Sub-Arctic regions, while Arctic *L. digitata* behaved similarly to cold-temperate populations. Our data suggest that a future increase in seawater temperatures may hamper the success of *H. nigripes* and favour *L. digitata* in Arctic environments.

Keywords: C:N-ratio; gametogenesis; growth rate; PAM fluorometry; sporophyte.

***Corresponding author: Kiara Franke**, Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany; and Current Address: Applied Ecology and Phycology, University of Rostock, Albert-Einstein-Straße 21, 18059 Rostock, Germany, E-mail: kiara.franke@uni-rostock.de
Daniel Liesner, Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany; and Department of Algal Development and Evolution, Max Planck Institute for Developmental Biology, Max-Planck-Ring 5, 72076 Tübingen, Germany
Svenja Heesch, Applied Ecology and Phycology, University of Rostock, Albert-Einstein-Straße 21, 18059 Rostock, Germany
Inka Bartsch, Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany. https://orcid.org/0000-0001-7609-2149

1 Introduction

Two digitate kelp species, *Laminaria digitata* (Hudson) J.V. Lamouroux and *Hedophyllum nigripes* (J. Agardh) Starko, S.C. Lindstrom *et* Martone are similar in their external morphology and they are part of marine forests in the shallow sublittoral of the Arctic (Dankworth et al. 2020; Longtin and Saunders 2016; Starko et al. 2019). They can be distinguished macroscopically by the presence or absence of mucilage ducts in their stipes and the season of fertility (Agardh 1868; Dankworth et al. 2020; Longtin and Saunders 2015). This had already been noted by Agardh (1868) who first described *H. nigripes* as *Laminaria nigripes* from Spitsbergen and compared it to various forms of European Arctic *L. digitata*.

As both species share the same habitat and as their unambiguous identification relies on molecular barcoding, the two species have been partially confused in the European Arctic for the past decades (e.g. Fredriksen et al. 2019; Lund 2014).

Meanwhile, it is known that *H. nigripes* is not present only in the European Arctic, but has a pan-Arctic distribution from the NE-Pacific (Cape Baele, BC, Canada) via the Canadian Arctic, the Bay of Fundy, USA (~45°N), and Greenland to Spitsbergen, Norway in the E-Atlantic (Dankworth et al. 2020; Guiry and Guiry 2021; Longtin and Saunders 2015; Pedersen 2011; Starko et al. 2019). Grant et al. (2020) investigated the phylogeography of *H. nigripes*, and their genetic studies suggest that the species evolved in the NE-Pacific. The genetic diversity of specimens in the Gulf of Alaska indicates the possible

existence of northern refugia during ice ages. However, it is uncertain whether N-Atlantic *H. nigripes* populations expanded from southern populations, or expanded their range from northern glacial refugial populations where the species survived (Grant et al. 2020). The exact southern distribution limits of *H. nigripes*, especially in the N-Atlantic, are unknown. In contrast, *L. digitata* has a pan-Atlantic to Arctic distribution, and its southern distribution limit correlates with the mean 19 °C August sea surface isotherm (van den Hoek 1982; Müller et al. 2009). The two species co-occur in the European Arctic and in the NW-Atlantic Sub-Arctic (e.g. Bay of Fundy; Dankworth et al. 2020; Fredriksen et al. 2019; Longtin and Saunders 2016).

Both species possess the heteromorphic, diplohaplontic life cycle typical for kelps, in which sporophytes must recruit from gametophytes (Lüning 1990; Martins et al. 2017; Oppliger et al. 2012). Kelp sporophytes generally have a narrower temperature window for growth and reproduction compared to gametophytes (e.g. Bartsch et al. 2008; tom Dieck 1992, 1993, Martins et al. 2017). Thermal characteristics of these fitness-related traits reveal the physiological temperature performance ranges of life stages and provide basic autecological information. A recent global overview and a series of global reports during the last decades showed that warming sea temperatures have direct and indirect effects on kelp abundance worldwide (Filbee-Dexter et al. 2019) and also in the Arctic (Bartsch et al. 2016; Krause-Jensen et al. 2020).

The temperature performance of *L. digitata* gametophytes and sporophytes is known for several N-Atlantic populations, showing a broad thermal performance range with evidence for slight local adaptation (e.g. Bolton and Lüning 1982; tom Dieck 1992, 1993; Liesner et al. 2020; Lüning 1980; Martins et al. 2017). However, no ecophysiological data currently exist for *H. nigripes*. In recent years, it became evident that a digitate kelp species had considerably increased its biomass at shallow areas in Kongsfjorden, western Spitsbergen, as a consequence of ocean warming (Bartsch et al. 2016). A follow-up study revealed that this increase was most probably due to *L. digitata* and not *H. nigripes* (Dankworth et al. 2020). Thus, we hypothesized that, despite sharing a similar habitat and morphology (Dankworth et al. 2020; Longtin and Saunders 2016), both species possibly do not share similar physiological traits and functions. In order to tackle this question, we wanted to compare thermal characteristics of fitness-related traits in two life cycle stages (gametophytes and sporophytes) of both species in laboratory experiments. The resulting comprehensive data were also used for the assessment of the current and potential future distribution of both species in a warming Arctic. In particular, we quantified gametogenesis, sporophyte recruitment and growth, optimal quantum yield and carbon and nitrogen content of sporophytes, as well as survival of both life cycle stages along temperature gradients, using several unialgal strains isolated from the European Arctic in Kongsfjorden, western Spitsbergen.

2 Materials and methods

2.1 Algal material

Clonal vegetative male and female gametophytes were isolated from four sporophyte individuals each of *Laminaria digitata* (AWI culture nos. 3465–3470, 3475, 3476) and *Hedophyllum nigripes* (AWI culture nos. 3501–3508) collected from Kongsfjorden, Spitsbergen in 2015 and 2016, respectively. Vegetative gametophytes were cultivated in sterile half-strength Provasoli enriched seawater (PES, Provasoli 1968; iodide enrichment according to Tatewaki 1966, double concentration of Na_2 glycerophosphate) at either 15 °C (*L. digitata*) or 10 °C (*H. nigripes*). Cultures were maintained at 3 µmol photons m^{-2} s^{-1} red light (LED Mitras lightbar daylight 150 controlled by ProfiLux 3, GHL Advanced Technology, Kaiserslautern, Germany), in a 16:8 h light:dark (LD) cycle.

2.2 Genetic confirmation of species identity

DNA was extracted from approximately 10 mg silica-dried gametophyte tissue using the Plant Nucleospin II extraction kit (Macherey-Nagel, Düren) according to the enclosed protocol, with an extended cell lysis step of 1 h. For the amplification of the mitochondrial cytochrome oxidase 1 gene (COI-5P) and the nuclear-encoded internal transcribed spacer (ITS) 1, polymerase chain reactions (PCRs) of 25 µl contained 8 µl water, 12.5 µl MyTaq™ premix (Bioline), 1 µl of each primer (COI-5P: GazF2 and GazR2, Lane et al. 2007; ITS: AFP2(F) and 5.8S1(R), Peters and Burkhardt 1998; biomers.net) and 2.5 µl DNA extract diluted with water (1:10 or 1:100) prior to amplification. All other methods including the PCR protocol, product purification, sequencing and sequence checks followed Heesch et al. (2016). Sequences were manually aligned using PhyDE-1 v0.9971 (Müller et al. 2010) and subjected to phylogenetic analyses, with methods given in Heesch et al. (2016, 2020). Strains with AWI culture nos. 3465–3476 were confirmed to belong to *L. digitata* (European nucleotide archive [ENA]/GenBank accession nos. for COI-5P: MW626932–MW626942; for ITS: MW616807–MW616813), while strains with AWI culture nos. 3501–3508 belonged to *H. nigripes* (ENA/GenBank accession nos. for COI-5P: MW626943–MW626946; for ITS: MW616814–MW616817). In addition, a female strain from Bamfield (Canada) originally isolated by L. Druehl in 1979 and studied in tom Dieck (1993) under the name of *Laminaria bongardiana* Postels *et* Ruprecht (= *Laminaria groenlandica sensu* Druehl, 1968; original culture no. 1110) was verified to belong to *H. nigripes* (AWI culture no. 3275, ENA/GenBank accession no. for COI-5P: MW626947; for ITS: MW616818).

2.3 Experiment 1: juvenile sporophytes

2.3.1 Pre-cultivation phase: In both species, gametophyte fertility was induced at 10 °C, 16:8 h LD, at 15 µmol photons m^{-2} s^{-1} white LED light in half-strength PES, following the method of Bartsch (2018). Juvenile sporophytes (2 cm long) were transferred to sterile aerated 5 l glass beakers at 40 µmol photons m^{-2} s^{-1}. Medium was changed either every two weeks (gametophyte phase) or weekly (sporophyte phase). For logistical reasons, experiments had to be performed consecutively under otherwise identical conditions. Therefore, *H. nigripes* sporophytes were first grown at 20 µmol photons m^{-2} s^{-1} to reduce growth rates, while the first experiment was conducted with *L. digitata*. The former were re-exposed to an irradiance of 40 µmol photons m^{-2} s^{-1} two weeks prior to the experiment, to allow for experimentation under comparable conditions.

2.3.2 Experimental set-up: Sporophytes were subjected to a temperature gradient (*L. digitata:* 0, 5, 10, 15, 18, 19, 20, 21, 22 ± 0.5 °C; *H. nigripes:* 0, 5, 10, 15, 16, 17, 18, 19, 20 ± 0.5 °C) for 14 days (day 0–14), followed by a post-cultivation phase (day 14–21) at 10 °C under identical irradiance conditions. For both species, nine sporophytes per replicate (n = 5) and temperature treatment were transferred into aerated glass beakers (1.8 l; half-strength PES). Sporophytes were acclimatized over six days (day −6 to 0; Supplementary Figure S1), before being exposed to the temperature gradient above. The last temperature step took place at day 0 for all treatments except for the control (10 °C), in order to start all experimental temperatures at the same time (Supplementary Figure S1). All temperatures were maintained either in walk-in constant cooling rooms (5–15 °C) or in water baths fitted with thermostats (Huber Variostat CC + Pilot ONE, Peter Huber Kältemaschinen GmbH, Offenburg, Germany). Medium was changed every 3–4 days. Thermal performance of sporophytes was determined by quantifying growth rates, carbon and nitrogen contents and optimal quantum yield (F_V/F_M) (Supplementary Table S1).

2.3.3 Growth: Sporophytes were photographed together with a calibration area, and their blade area was determined via image analysis (Image J: Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA, 1997–2018) every 3–4 days. Linear relative growth rates (RGR) were calculated according to the following formula:

$$\mathrm{RGR}\left[\%\ \ \mathrm{d}^{-1}\right] = \frac{\frac{\mathrm{area}_{\mathrm{final}} - \mathrm{area}_{\mathrm{initial}}}{\mathrm{area}_{\mathrm{initial}}}}{\mathrm{T}} \times 100,$$

where T is time (days). In order to discriminate between acclimation of growth rate over time and overall growth rate, RGR was calculated between consecutive time points and over the 14-day period separately. For a direct comparison of both species, RGR were normalized to the respective maximum value per species, which was set as 100% (called: Standardized GR).

2.3.4 F_V/F_M: The *in vivo* chlorophyll *a* fluorescence of photosystem II of dark-acclimated (5 min) *L. digitata* and *H. nigripes* sporophytes was measured using pulse amplitude modulated (PAM) fluorometry (Mini PAM, Walz, Effelrich, Germany) as described by Schreiber et al. (1986). I-PAM images (Imaging PAM, Walz, Effelrich, Germany) were used as a qualitative measure of the overall stress response of sporophytes.

2.3.5 Carbon and nitrogen contents: Three sporophytes per replicate were pooled, snap-frozen in liquid nitrogen and preserved at −80 °C at day 0, 7 and 14. Deep frozen sporophytes were freeze-dried (CHRIST, ALPHA 1–4 LD plus, Osterode am Harz, Germany) for 24 h, and dry weight (DW) was measured. Samples of 2–3 mg were combusted at 1000 °C in a CN-Elementar analyser (Euro EA 3000: Euro Vector, Pavia, Italy) with acetanilide as standard.

2.4 Experiment 2: gametophytes and reproduction success

2.4.1 Experimental set-up: Stock gametophyte cultures of *H. nigripes* were acclimatized for two weeks at 15 °C before the start of the experiment. A gametophyte stock solution was prepared separately, for each species, by pooling even amounts of vegetative male and female gametophytes, slightly squashing the material in a sterile mortar, sieving it through a sterile 100 µm mesh and suspending it in half-strength PES. Because of contamination, AWI culture 3508 (female of *H. nigripes*) was excluded from the gametophyte experiments.

For each species, this stock solution was used to sow a target density of 1000 gametophytes cm^{-2} into four replicate petri dishes containing 100 ml of half-strength PES. The mean final densities were 810 ± 178 and 1028 ± 188 gametophytes cm^{-2} for *L. digitata* and *H. nigripes*, respectively, and were statistically different (t-test, $p < 0.0005$). Gametophytes were acclimatized over six days (day −6 to 0, Supplementary Figure S2) at 3 µmol photons m^{-2} s^{-1} white LED light, 16:8 h LD cycle. At day 0, irradiance was increased to 15 µmol photons m^{-2} s^{-1}. The last temperature step took place at day 0 for all treatments, except for the control (15 °C), in order to start all experimental temperatures at the same time (Supplementary Figure S2; *L. digitata:* 0, 5, 10, 15, 20, 22, 23, 24, 25 ± 0.5 °C, *H. nigripes:* 0, 5, 10, 15, 18, 19, 21, 22 ± 0.5 °C). One replicate of *H. nigripes* at 15 °C had to be excluded from analyses due to contamination. After two weeks, the temperature gradient was followed by a post-cultivation phase: Gametophytes exposed to ≤10 °C were kept in the same conditions for another week (day 14–21) to quantify sporophyte recruitment, while gametophytes exposed to ≥15 °C were kept at 15 °C to follow recovery for two weeks (day 14–28; Supplementary Figure S2). Before post-cultivation, 50 ml of the medium was exchanged. After two weeks of post-cultivation, the 15, 24 and 25 °C (*L. digitata*) and 15, 21 and 22 °C (*H. nigripes*) treatments received iron-free half-strength PES and were kept at 15 °C under red light to promote vegetative gametophyte growth (Iwai et al. 2015) for long-term post-cultivation (after day 28). Keeping the gametophytes under red light is a standard way for cultivating gametophytes in a vegetative stage, as blue light included in regular white LED light initiates gametogenesis and reproduction (Bartsch et al. 2016; Lüning and Dring 1972; Redmond et al. 2014). Gametogenesis is easily induced even in vegetative gametophyte cultures that have been kept over decades, under red light conditions (e.g. Martins et al. 2019).

2.4.2 Quantification of gametogenesis: The relative abundance of three ontogenetic stages (vegetative gametophytes (with or without oogonia); gametophytes with released eggs; gametophytes with sporophytes; Martins et al. 2017) was counted at day 7, 14 and 21 using an Olympus CKX41 inverted microscope (Olympus Co., Tokyo, Japan). At day 0, approximately 200 female gametophytes per replicate (n = 4)

were counted and related to the petri dish area. At each subsequent counting day, ontogenetic stages were quantified in this area.

2.4.3 Temperature tolerance of male and female gametophytes: To quantify the temperature tolerance of gametophytes, the relative abundance of living gametophytes of both sexes was recorded at day 7, 14 and 21 and the sex ratio (female/male) was calculated. Gametophyte cells were considered alive when no plasmolysis had taken place and at least one cell per fragment was pigmented.

2.5 Statistics

Statistical analyses were performed with Statistica 8.0 (Statsoft Inc., 2007, Minneapolis, USA). Following confirmation of variance homogeneity (Levene's test, $\alpha = 0.05$), one- or two-way analysis of variance (ANOVA) were performed and followed by a Tukey's post hoc test. For data with heterogeneous variance distribution, non-parametric Friedman tests for temperature effects and Kruskal–Wallis tests with multiple *p*-value comparison for time effects were applied followed by Bonferroni correction.

2.6.1 Experiment 1: juvenile sporophytes: *Hedophyllum nigripes* sporophytes exposed to 19 and 20 °C died during the experimental phase and were therefore excluded from statistical analyses. Standardized growth rate (GR) of both species (0–18 °C) were compared in a two-way ANOVA with 'temperature' and 'species' as fixed factors. For the interspecies comparison, only the temperature treatments common to both species (0–18 °C) were analysed. Standardized GR was analysed in a one-way ANOVA for both species separately after 14 days. RGR over time (experimental phase and recovery) were grouped by temperatures and analysed in Friedman tests in each species separately. F_V/F_M of both species at day 14 at 0–18 °C was compared in a two-way ANOVA with 'temperature' and 'species' as fixed factors. As variances were heterogeneous, the significance level was decreased to alpha = 0.01 to reduce the probability of false positive reports. Due to heterogeneity, the response of F_V/F_M over time and temperature was tested separately, applying non-parametric Friedman tests for both species and for each temperature. The temperature × time interaction for F_V/F_M of each species was analysed with Kruskal–Wallis tests. Differences in F_V/F_M between responses on day 14 and after recovery were analysed by non-parametric Friedman tests. Carbon and nitrogen contents and C:N-ratios at day 0 and 14 of both species were compared in *t*-tests.

2.6.2 Experiment 2: gametophytes and reproduction success: Gametophytes of *L. digitata* exposed to 24 and 25 °C, and of *H. nigripes* exposed to 21 and 22 °C died during the experimental phase. These temperatures were therefore excluded from the analyses. Gametophyte density of both species at day 0 was compared in a *t*-test. Survival over time was tested in two separate repeated measures (RM) ANOVAs for each species separately. The recovery of *L. digitata* was determined in an RM ANOVA and for *H. nigripes* in a non-parametric Friedman test separated by temperatures. The change of the sex ratio of gametophytes over time was tested in non-parametric Friedman tests (*L. digitata*) or an RM ANOVA (*H. nigripes*). Sporophyte recruitment was quantified as percentages of gametophytes bearing sporophytes and was normalized to the respective maximum value per species (=100%). Values were analysed in a two-way ANOVA for day 14. As the homogeneity of variance was met only partially, the significance level was decreased to alpha = 0.01 to reduce the probability of false positive reports.

3 Results

3.1 Experiment 1: juvenile sporophytes

3.1.1 Temperature tolerance and growth

Standardized GR comparisons revealed temperature × species interactions at 0, 5, 10, 15 and 18 °C (two-way ANOVA, $F_{4,40} = 27.3$, $p < 0.0001$; Supplementary Table S2): At 15 and 18 °C, *L. digitata* grew better than *H. nigripes*, while *H. nigripes* grew better at 0, 5 and 10 °C compared to *L. digitata* (Figure 1). In both species, growth responded to temperature (two-way ANOVA, $F_{4,40} = 39.3$, $p < 0.0001$; Supplementary Table S2; Standardized GR).

Laminaria digitata sporophytes growth was maximal at 15 °C (Figure 1; RGR = 9.2 ± 1.4% d^{-1}; data not shown) and higher than in all other temperatures (Tukey's post hoc test, $p < 0.02$). Standardized GR at 0 and ≥20 °C were less than 20% of the maximum (Figure 1). Moreover, stress was indicated by reduced chlorophyll fluorescence at ≥20 °C (I-PAM images, Supplementary Figure S3) and reduced RGR (Figure 2) as well as bleached distal areas at ≥21 °C (Figure 3). RGR became negative after 14 days at 0 and ≥18 °C (Figure 2); a decrease over time was significant only for 18 °C (Friedman test, $\chi^2_{3,5} = 11.9$, $p = 0.008$) and 19 °C (Friedman test,

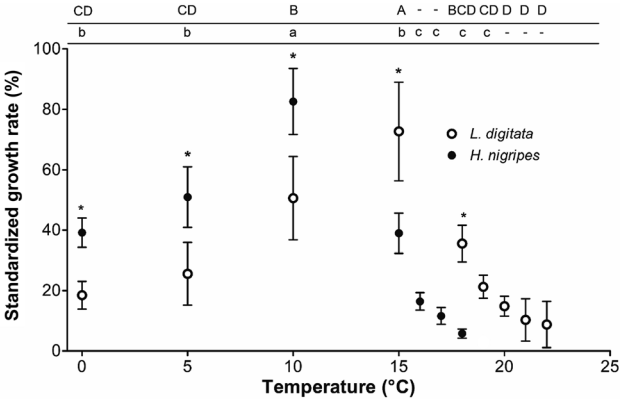

Figure 1: Standardized growth rates (GR) based on surface area (%) of *Laminaria digitata* (white dots) and *Hedophyllum nigripes* (black dots) sporophytes over two weeks in a temperature gradient ($n = 5$, mean ± SD). Different letters denote significant differences within each species (ANOVA with Tukey's post hoc test: $\alpha < 0.05$, A–D = *L. digitata*; a–c = *H. nigripes*). Asterisks indicate significant differences between standardized GR of *L. digitata* and *H. nigripes* (two-way ANOVA with Tukey's post hoc test).

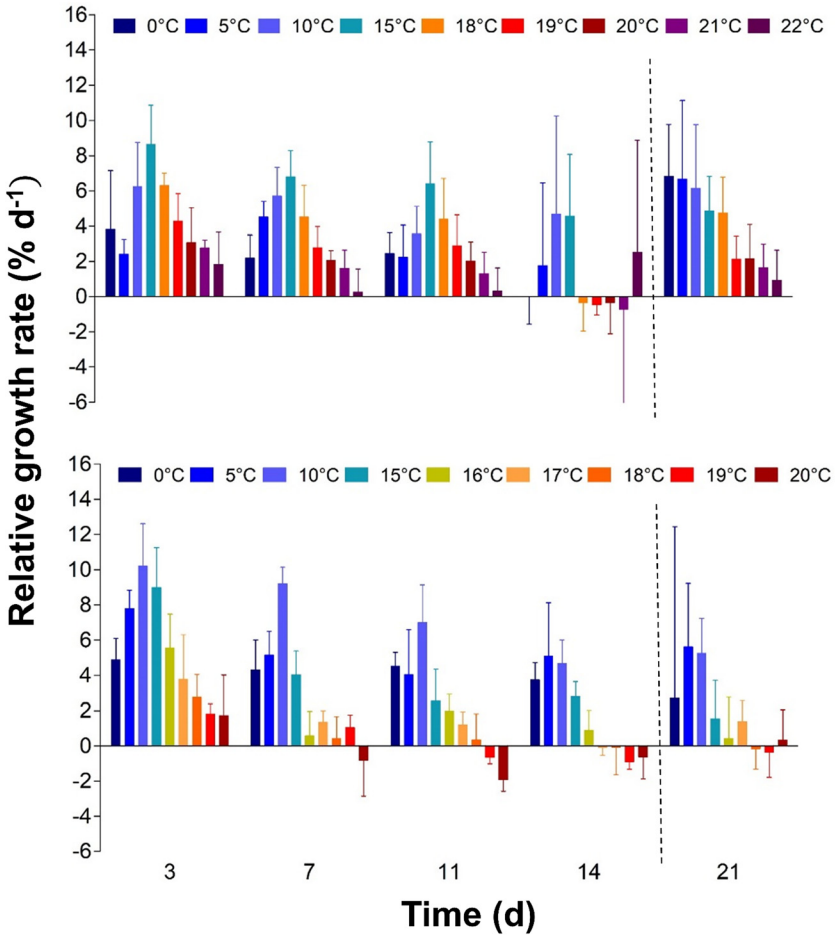

Figure 2: Relative growth rates (RGR; % d^{-1}) of *Laminaria digitata* (top) and *Hedophyllum nigripes* (bottom) sporophytes in a temperature gradient over the experimental time (14 days; left side of the dotted line) and recovery at 10 °C (one week; right side of the dotted line; $n = 5$, mean ± SD). Each value denotes the RGR between the indicated time point and the measuring day before.

$\chi^2_{3,5} = 10.7$, $p = 0.01$). After one week of recovery at 10 °C, RGR increased only in 0, 5 and 18 °C treatments (Friedman test, $\chi^2_{1,5} = 5.0$, $p = 0.025$) by up to 6.8% d^{-1} (0 °C) (Figure 2).

Hedophyllum nigripes sporophytes had a growth maximum at 10 °C, which was thereby 5 °C lower than the maximum of *L. digitata* (Figure 1; RGR = 4.8 ± 0.2% d^{-1}; data not shown) where growth was higher than in all other temperatures (Tukey's post hoc test, $p < 0.0002$). Standardized GR at 0 °C also did not fall below 20% of the maximum, in contrast to *L. digitata*. At temperatures ≥16 °C, however, standardized GR fell below the 20% level (Figure 1), accompanied by signs of blade bleaching (Figure 3, I-PAM images, Supplementary Figure S3). At 20 °C, RGR became negative already after three days (Figure 2). After one week of recovery at 10 °C, RGR increased only in 17 °C treatments (1.4% d^{-1}, Friedman test, $\chi^2_{1,5} = 5$, $p = 0.03$).

3.1.2 F_V/F_M

Comparison of F_V/F_M of both species (0, 5, 10, 15 and 18 °C) revealed temperature × species interactions (two-way ANOVA, $F_{4,40} = 14.9$, $p < 0.0001$) and temperature effects (two-way ANOVA, $F_{4,40} = 13.3$, $p < 0.0001$). The mean F_V/F_M was 0.72 for both species at day 0. In *L. digitata* sporophytes, F_V/F_M was maximal at 10 and 15 °C with an increase of 7% at day 14 ($F_V/F_M = 0.74 \pm 0.01$; Supplementary Tables S3 and S4, Figure 4). At 21 °C ($F_V/F_M = 0.63 \pm 0.05$) and 22 °C ($F_V/F_M = 0.34 \pm 0.08$), F_V/F_M decreased over time by 15 and 54%, respectively (Figure 4, Supplementary Table S4). Following the 0 °C treatment, F_V/F_M of sporophytes increased after one week of recovery at 10 °C (Friedman test, $\chi^2_{1,5} = 5$, $p = 0.02$). In sporophytes from the 10, 15, 19 and 20 °C treatments, F_V/F_M decreased by up to 10% during the recovery period (Friedman test, 10 °C: $\chi^2_{1,5} = 1.8$, $p < 0.05$; 15, 19, 20 °C: $\chi^2_{1,5} = 5$, $p = 0.03$). Sporophytes from 21 to 22 °C showed no recovery. Compared to F_V/F_M of *L. digitata* at 0 °C, *H. nigripes* continuously showed 8% higher mean values at 0 °C than *L. digitata* (Tukey's post hoc test, $p < 0.02$). At 17 and 18 °C, F_V/F_M in *H. nigripes* decreased by 9% (at day 14, $F_V/F_M = 0.63 \pm 0.05$). After one week of recovery at 10 °C, *H. nigripes* sporophytes from 0 to 18 °C treatments stayed the same as during the treatment (Figure 4).

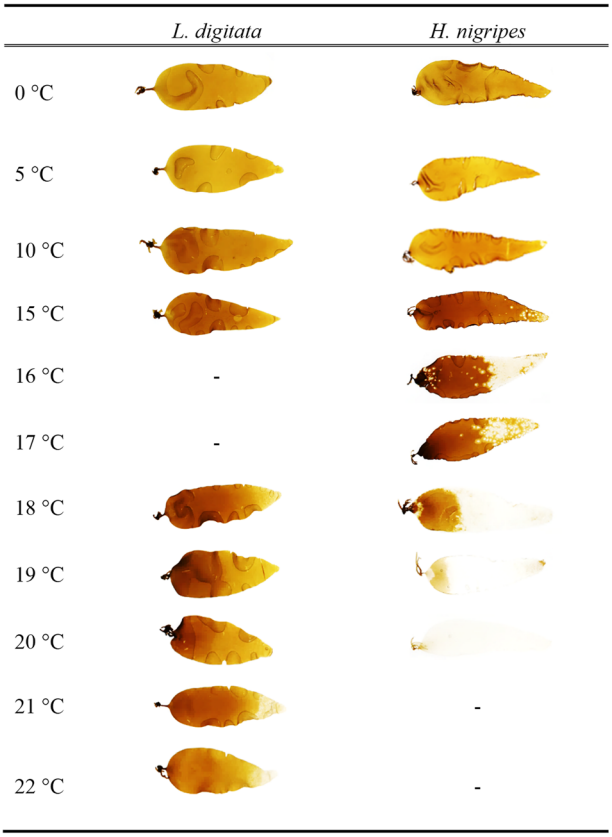

Figure 3: Photographic documentation of *Laminaria digitata* and *Hedophyllum nigripes* sporophytes exposed to a temperature gradient after post-cultivation at 10 °C. Images are not to scale. Triangular cuts marked individual sporophytes per replicate.

3.1.3 Carbon and nitrogen content

Laminaria digitata showed a 10% higher carbon content at day 0 (0–18 °C; *t*-test, $p = 0.05$, Table 1) compared to *H. nigripes* (*t*-test, $p = 0.007$), while the nitrogen content was 100% higher in *H. nigripes* compared to *L. digitata* (*t*-test, $p < 0.0001$) during the whole experiment (Table 1). Consequently, the C:N-ratio of *L. digitata* was 40% higher than that of *H. nigripes* after 14 days (*t*-test, $p < 0.0001$, Table 1).

3.2 Experiment 2: gametophytes and reproduction success

3.2.1 Temperature tolerance and survival

At days 7 and 14, gametophyte density was affected by a temperature × time interaction in *L. digitata* (0–23 °C; RM ANOVA, $F_{12,42} = 12$, $p = 0.01$, Supplementary Table S5, Figure 5). After seven days, the gametophyte density decreased by 48 and 40% at 0 and 23 °C, respectively (480 ± 92 and 637 ± 116 gametophytes cm^{-2}, respectively; Tukey's post hoc test, $p < 0.03$). At 25 °C, all gametophytes died after one week (Figure 5A), and at 24 °C after two weeks (Figure 5C). After two weeks of post-cultivation at 15 °C, gametophytes from 24 and 25 °C treatments showed no recovery. However, after seven weeks, 0.2% (1.4 ± 0.8 gametophytes cm^{-2}) of the initial gametophyte number recovered from the 24 °C treatment.

In *H. nigripes* only time affected the gametophyte density in all temperatures (0–19 °C; RM ANOVA, $F_{2,34} = 35.7$, $p < 0.0001$, Supplementary Table S5, Figure 5). The gametophyte density (0–19 °C) decreased from initially 1113 ± 201 gametophytes cm^{-2} to 764 ± 111 gametophytes cm^{-2} at day 14, which was, however, not significant (Figure 5). At 21 and 22 °C, all gametophytes died after 14 days. After two weeks of post-cultivation at 15 °C, gametophytes showed no recovery. However, in long-term culture under red light at 15 °C (one year), 0.08% (0.4 ± 0.03 gametophytes cm^{-2}) of the initial gametophyte number recovered from the 21 °C treatment.

3.2.2 Sex ratio

All replicates of both species contained approximately two-fold fewer female than male gametophytes (ratio: 0.4 ± 0.1, day 7, data not shown). In both species, the abundance of females further decreased with increasing temperatures over time (ratio: 0.24 ± 0.03, day 14, Figure 6). In *L. digitata*, sex ratio decreased between day 0 and 14 (Friedman test: 0, 5, 10, 15, 22 °C: $\chi^2_{2,4} = 6.5$. $p < 0.04$; 20, 21, 23 °C: $\chi^2_{2,4} = 8.0$, $p < 0.02$). The strongest decrease of 85% (to 0.05 ± 0.01) occurred at 23 °C, indicating that many more females than males had died at this temperature. Likewise, in *H. nigripes*, there was a temperature × time interaction on the sex ratio (RM ANOVA, $F_{5,17} = 37.1$, $p < 0.0001$, Supplementary Table S6). The sex ratio was decreasing at 0, 18 and 19 °C between day 7 and 14 (Tukey's post hoc test: $p < 0.0002$), while it kept stable at 5–15 °C.

3.2.3 Development of ontogenetic stages

Both species recruited sporophytes between 0 and 15 °C within 14 days, but not at higher temperatures (Figure 7). There was a temperature × species interaction for sporophyte recruitment (ANOVA, $F_{3,23} = 20.9$, $p < 0.0001$, Supplementary Table S7). *Laminaria digitata* optimally recruited between 5 and 15 °C (80–90%) and *Hedophyllum nigripes* between 0 and 10 °C (70–80%). *Hedophyllum nigripes* recruited more sporophytes at 0 °C

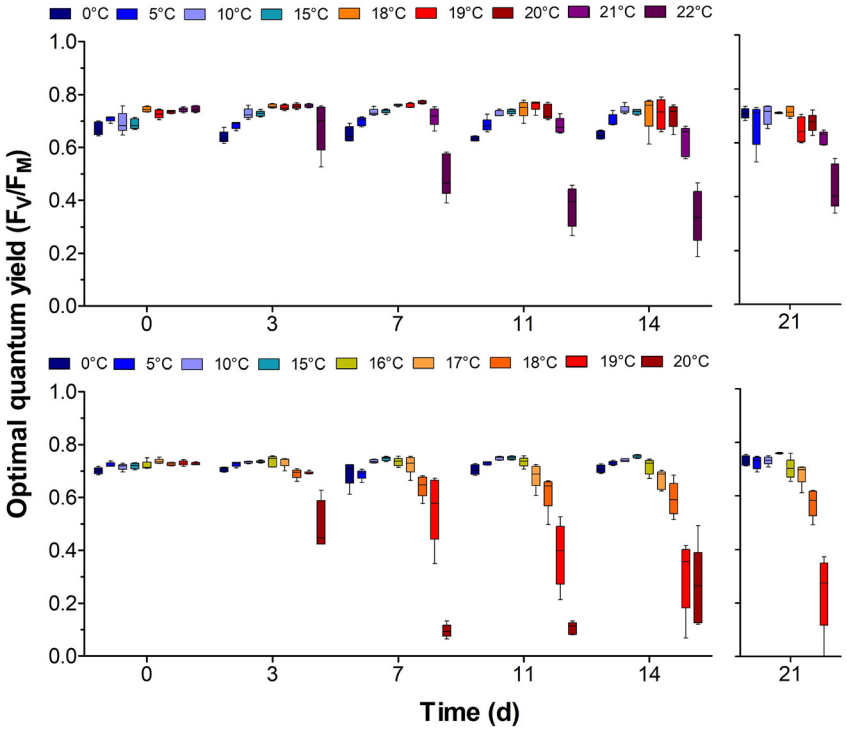

Figure 4: Optimal quantum yield (F_V/F_M) of *Laminaria digitata* (top) and *Hedophyllum nigripes* (bottom) sporophytes in a temperature gradient (two weeks; left graph) and post-cultivation at 10 °C (one week; right graph). Horizontal lines represent the median; boxes, the interquartile range; whiskers, 1.5× of inter-quartile range (n = 5).

Table 1: Carbon content (mmol C g^{-1} DW), nitrogen content (mmol N g^{-1} DW) and carbon:nitrogen (C:N) ratio of *Laminaria digitata* and *Hedophyllum nigripes* at day 0 (T0) and day 14 (T14) (n = 5; mean ± SD).

	Carbon		Nitrogen		C:N-ratio	
	T0	T14	T0	T14	T0	T14
L. digitata	29 ± 0.62	29.9 ± 1.26	1.2 ± 0.06	1.3 ± 0.13	23.4 ± 1.23	22.2 ± 0.59
H. nigripes	27 ± 2.4	28 ± 5.46	2.4 ± 0.27	2.2 ± 0.51	11.2 ± 0.78	13.3 ± 1.21

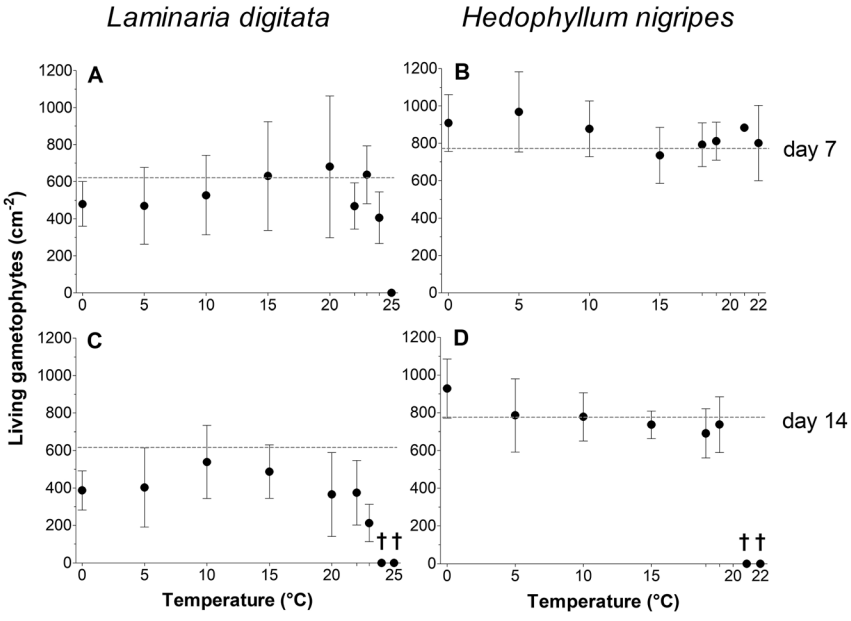

Figure 5: Density of gametophytes of *Laminaria digitata* (A, C) and *Hedophyllum nigripes* (B, D) at day 7 (A, B) and day 14 (C, D) in temperature gradients between 0 and 25 °C (*L. digitata*) and 22 °C (*H. nigripes*) (n = 3–4, mean ± SD). Broken horizontal lines show the mean initial gametophyte density for each species after the acclimatization phase (day 0). †All gametophytes died.

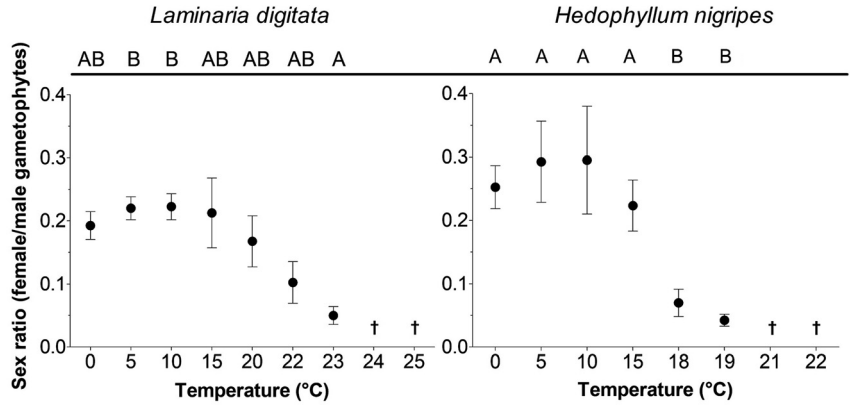

Figure 6: Sex ratio (female:male) of gametophytes of *Laminaria digitata* (left) and *Hedophyllum nigripes* (right) after 14 days in temperature gradients between 0 and 25 °C (*L. digitata*) and 22 °C (*H. nigripes*) ($n = 4$, mean ± SD). Different letters denote significant differences among temperatures within each species (*L. digitata*: Kruskal–Wallis test with multiple *p*-value comparison; *H. nigripes*: one-way ANOVA with Tukey's post hoc test). Please note that the marked deviation from an expected initial 50:50 ratio was due to applied seeding methods. †All gametophytes died.

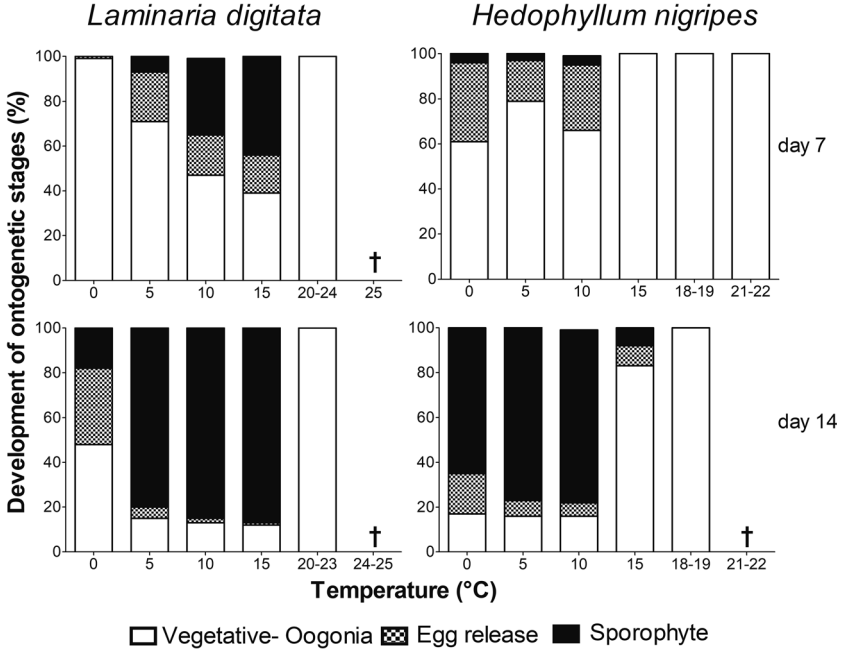

Figure 7: Effect of temperature on the relative abundance of different ontogenetic stages during gametogenesis of *Laminaria digitata* (left) and *Hedophyllum nigripes* (right) in a temperature gradient after seven days (above) and 14 days (below; mean of $n = 3–4$; SD not shown for clarity). Only the most developed stage was counted per female gametophyte. †All gametophytes died.

than *L. digitata* (Tukey's post hoc test: $p < 0.001$), while *L. digitata* recruited more sporophytes at 15 °C than *H. nigripes* (Tukey's post hoc test: $p < 0.004$). At 5 and 10 °C, sporophyte recruitment of both species was the same. While *L. digitata* showed the first sporophytes at 5–15 °C after one week (10–40%), only a few sporophytes were apparent at 0–10 °C in *H. nigripes* at the same time (~5%) (Figure 7), showing a delay in recruitment in *H. nigripes*.

4 Discussion

The present study clearly demonstrates that co-occurring Arctic *Laminaria digitata* and *Hedophyllum nigripes* from Spitsbergen possess very dissimilar temperature characteristics and thereby possibly occupy different ecological niches. Upper temperature survival limits for sporophytes and gametophytes as well as optima for sporophyte growth, gametophyte survival and sporophyte recruitment differed in both kelps by 4–5 °C, with *L. digitata* showing more temperate characteristics than *H. nigripes* (Table 2). For the first time it becomes evident that *H. nigripes* is a species with an Arctic to Sub-Arctic temperature imprint (*sensu* Wiencke et al. 1994). Overall, *H. nigripes* has a better performance at 0 °C than *L. digitata*, and prefers cold temperatures between 5 and 10 °C for optimum growth and sporophyte recruitment. Over a two-week exposure, *H. nigripes* sporophytes can tolerate a maximum of 18 °C and gametophytes can tolerate 19–21 °C. Thereby, its temperature performance is comparable to the Arctic endemic kelp *Laminaria solidungula* J. Agardh, which shows a similar growth optimum at 5–10 °C, while sporophytes

Table 2: Temperature tolerance of *Laminaria digitata* (*Ldig*) and *Hedophyllum nigripes* (*Hnig*) gametophytes (Game) and sporophytes (Sporo) determined after two weeks of exposure to the given temperatures and subsequent two-week post-cultivation at 10 °C and long-term culture under red light at 15 °C (post-cult.).

Species		Exp. time	Temperature (°C)														UST (°C)
			0	5	10	15	16	17	18	19	20	21	22	23	24	25	
Ldig	Game	One week	V	S	S	S					V	V	V	V	V	––	24
		Two weeks	S	S	S	S					V	V	V	V	––	––	23
		Post-cult.	S	S	S	S					V	V	V	V	R	––	24
	Sporo	One week	X	X	X	X			X	X	X	X	X				22
		Two weeks	X	X	X	X			X	X	X	X	X				22
		Post-cult.	X	X	X	X			X	X	X	X	X				22
Hnig	Game	One week	S	S	S	V			V	V		V	V				22
		Two weeks	S	S	S	S			V	V		––	––				19
		Post-cult.	S	S	S	S			V	V		R	––				21
	Sporo	One week	X	X	X	X	X	X	X	X	––						19
		Two weeks	X	X	X	X	X	X	X	––	––						18
		Post-cult.	X	X	X	X	X	X	X	––	––						18

UST, upper survival temperature; V, vegetative gametophytes; S, sporophyte recruitment; X, survival; ––, no survival; R, recovery after long term post-cultivation; Exp. time, exposure time.

survive 16 °C and gametophytes survive 19–20 °C (Bolton and Lüning 1982; tom Dieck 1992, 1993; Roleda 2016). Arctic *L. digitata* has a reduced performance at 0 °C compared to *H. nigripes*, and sporophyte growth and recruitment are optimal at 10–15 °C. Arctic *L. digitata* sporophytes survive 21–22 °C and gametophytes survive 23–24 °C for two weeks, which is similar among populations along the latitudinal distribution gradient in the NE-Atlantic (Liesner et al. 2020 and references therein; Schimpf 2021). Therefore, *L. digitata* is a typical representative of a cold-temperate seaweed species (Wiencke et al. 1994).

In both kelp species, all replicates showed a strong artificial unbalanced sex ratio towards male gametophytes despite the standardized method of squashing, sieving and sowing vegetative-grown gametophyte material (Bartsch 2018). This does not occur when initiating experiments from meiospores (Oppliger et al. 2012). We assume that the male gametophytes more easily fragmented into smaller pieces than females and thus unintentionally were enriched in the seeding stock solutions. Nevertheless, it became evident that female gametophytes had a lower survival capacity than males at high temperatures, leading to a further decrease in sex ratio with increasing temperature. This confirms previous reports indicating that male kelp gametophytes may survive 1–2 °C higher temperatures than females (Bolton and Lüning 1982; tom Dieck 1993; Oppliger et al. 2012). After two experimental weeks, the observed low sex ratio at 0–15 °C was likely a result of sporophyte recruitment at these temperatures, which diminished the number of female gametophytes.

In addition to their temperature performance, both species showed major differences in their ability to store nitrogen under replete nutrient conditions when cultivated in half-strength PES (274 µmol NO_3^- l^{-1}; Sarker et al. 2013). Interspecific differences in nutrient uptake ability have also been shown for other Polar seaweeds (Roleda and Hurd 2019). Despite a high concentration of nitrate in the medium, *L. digitata* sporophytes had a C:N-ratio of >20. This comparatively high C:N-ratio exceeds the mean value for temperate and tropical macroalgae and is normally more indicative of nutrient-poor conditions (Atkinson and Smith 1983). In contrast, *H. nigripes* had approximately 40% lower C:N-ratios of <14 irrespective of temperature, suggesting an optimal utilization of external nitrogen sources in this species (Gordillo et al. 2006). This might represent an ecological advantage of *H. nigripes* over *L. digitata* in Arctic environments which are generally nutrient poor and even more so from late spring onwards following the phytoplankton spring bloom (Gordillo et al. 2006; Piquet et al. 2014).

In order to better contrast the temperature performance of the two species and their intraspecific differences, the 'sufficient growth' concept of Cambridge et al. (1984) is helpful. These authors defined the temperature which allows 20% growth of the maximum as the marginal temperature allowing sufficient growth (or reproduction) for long-term survival of a population (Cambridge et al. 1984). In southern isolates of *L. digitata*, sporophytes grew at 0 and also 20–21 °C with 25–50% of the maximum (Bolton and Lüning 1982; tom Dieck 1992) while the

Spitsbergen material, used here, grew with less than 17% of the maximum at ≥20 °C. Although Spitsbergen *L. digitata* sporophytes looked superficially healthy at 22 °C, even after two weeks with only a few bleached areas, I-Pam images revealed that sporophytes were considerably stressed at ≥19 °C. F_V/F_M at these temperatures did not recover in post-cultivation, nor did sporophytes resume growth after transfer into optimum temperatures. The Spitsbergen *L. digitata* population may thereby belong to an ecotype that is slightly more susceptible to high temperatures than southern European populations and supports the existence of local adaptation towards high temperatures in the species (King et al. 2019; Liesner et al. 2020). There is also subtle evidence for ecotypic variation in *L. digitata* gametophytes, but only at the low temperature range. The upper temperature tolerance of *L. digitata* gametophytes is 23 °C and was stable in all investigated populations from the Arctic to the southern distribution limit (tom Dieck 1993; Lüning 1980; Schimpf 2021). But North Sea gametophytes showed a much reduced fertility at 1 °C (tom Dieck 1992), while it was relatively high (20%) at 0 °C in our Spitsbergen material. Similarly, sporophyte recruitment of Spitsbergen gametophytes was slightly better at low temperatures (5 °C) than for North Sea material (Martins et al. 2020).

In contrast to *L. digitata*, *H. nigripes* sporophytes grew with approximately 40% of the maximum rates at 0 °C but only with low rates at 16 °C (18%), supporting its affinity for cold Arctic environments. The general cold-adaption of *H. nigripes* sporophytes was also apparent in photosynthetic parameters. While F_V/F_M of *L. digitata* decreased at low temperatures ≤5 °C, F_V/F_M stayed high and stable in *H. nigripes* at 0–5 °C but dropped at ≥17 °C, concomitant to the reduced growth performance. Most probably an extended period of 15 °C is already stressful for *H. nigripes* sporophytes as photosynthetic inhibition already became visible in I-PAM images after two weeks exposure at 15 °C (Supplementary Figure S3).

The southern distribution limit of *H. nigripes* in the Atlantic is still unknown, but our physiological data may allow assumptions about its potential occurrence along the European coastline. In the western Atlantic, the southernmost records of *H. nigripes* are from the Lepreau region in the Bay of Fundy, where monthly snapshot measurements recorded a minimum sea surface temperature (SST) of 0.45 °C and a maximum SST of 14.5 °C (2002–2009; Assis et al. 2017; Bosch 2020; Tybergbein et al. 2012). This may represent a southern growth limit for sporophytes which drastically reduce their growth at >15 °C over 14 days (this study). In the NE-Pacific, the southernmost records for *H. nigripes* are from Cape Beale and Bamfield, British Columbia (as *Laminaria bongardiana* in tom Dieck 1993; Starko et al. 2019). In Bamfield, monthly snapshot measurements recorded a minimum SST of 7.8 °C and a maximum SST of 14.2 °C (2002–2009; Assis et al. 2017; Bosch 2020; Tybergbein et al. 2012). As *H. nigripes* showed highest gametophyte reproduction success at 0–10 °C, this may indicate that the southern distribution limit in the NE-Pacific is set by temperatures that enable sufficient sporophyte recruitment from gametophytes and in addition allow for sufficient sporophyte growth. Following the phytogeographic distribution concept of van den Hoek (1982), locations with winter SST < 10 °C and summer SST not surpassing 15 °C would allow for sufficient sporophyte recruitment and sufficient growth (similarly as around Bamfield), and thus would provide appropriate temperature conditions for this species.

We re-investigated the identity of a '*L. bongardiana*' culture that had been collected by L. Druehl from Bamfield, Vancouver Island, Canada in 1979 and was investigated by tom Dieck (1993). Barcode analysis of two genetic makers (COI-5P and ITS1) revealed its identity as *H. nigripes*. Also, the '*L. bongardiana*' cultures investigated by tom Dieck (1992), were most likely *H. nigripes*, as they were collected at the same time. Small intraspecific differences in temperature responses were apparent between the Bamfield and Spitsbergen isolates, with the Bamfield material being slightly warmer adapted (tom Dieck 1992, this study). The upper two-week temperature tolerance of Bamfield gametophytes was 20–21 °C (tom Dieck 1993) while Spitsbergen isolates survived 19 °C, but recovered with 0.08% from 21 °C treatments in long-term post cultivation. Similarly, juvenile sporophytes from Bamfield showed slightly higher affinities to warm temperatures than Spitsbergen material. Maximum growth was at 10 °C with 81 and 75% of the maximum rates at 5 and 15 °C, respectively (tom Dieck 1992), while Spitsbergen sporophytes also grew maximally at 10 °C but significantly lower at 15 °C than at 5 °C, and the relative growth performance at 0 °C was better than in the Bamfield material. Although, upper survival temperature of sporophytes from both locations was 18 °C, the Spitsbergen material suffered considerably at temperatures ≥16 °C (this study: Supplementary Figure S3; tom Dieck 1992). The differences in temperature adaptation may be a reflection of the slight genetic differences between investigated *H. nigripes* populations: while COI haplotypes from Svalbard in the NE-Atlantic differ by 1–2 mutations from NW-Atlantic material, the latter also differs by 1–2 mutations from NE-Pacific material (Grant et al. 2020).

5 Conclusion

A recent pan-Arctic review compiled the scattered information on trends in macrophyte abundance during recent decades. Most of the 38 sites indicated that macrophyte abundance, species richness and/or productivity is increasing in the Arctic (Krause-Jensen et al. 2020) with medium confidence Intergovernmental panel on climate change (IPCC) confidence scale, Shapiro et al. 2010). At our collection site in Kongsfjorden, especially *Laminaria digitata* has become very dominant in shallow water during recent decades (Bartsch et al. 2016; Dankworth et al. 2020). Our data suggest that *L. digitata* has the long-term potential of replacing *Hedophyllum nigripes*, which probably was the overlooked but prevalent digitate kelp species around Spitsbergen (Fredriksen et al. 2019; Lund 2014). Kongsfjorden (Spitsbergen) showed a minimum SST of –0.6 °C and a maximum SST of 3.7 °C between 2002 and 2009 (Assis et al. 2017; Bosch 2020; Tybergbein et al. 2012), and future predictions assume a minimum SST of 1.6 °C and a maximum SST of 10.5 °C in 2100 (representative concentration pathway [RCP] 8.5; Assis et al. 2017; Bosch 2020; Tybergbein et al. 2012). Although, *L. digitata* and *H. nigripes* overlap in their thermal range for sporophyte recruitment (0–15 °C), *L. digitata* showed a better performance in the upper range (5–15 °C). We therefore expect, that this species will be better adapted to future increased temperatures than *H. nigripes*. This hypothesis is supported by *in situ* data from the western Atlantic, where *H. nigripes* and *L. digitata* co-occur in the Bay of Fundy, and provide evidence of ongoing competition processes in relation to environmental temperatures. The abundance of *H. nigripes* decreased, and it was replaced by *L. digitata* after a period of warm winters surpassing 8 °C (Longtin and Saunders 2016). In times of global warming, and especially a fast-warming Arctic, we thus expect *H. nigripes* to retract to more northern locations with unknown consequences for ecosystem functioning.

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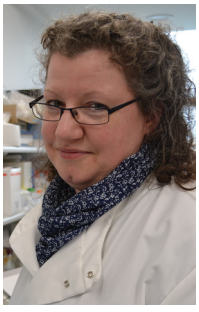
Bionotes

Kiara Franke
Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
Applied Ecology and Phycology, University of Rostock, Albert-Einstein-Straße 21, 18059 Rostock, Germany
kiara.franke@uni-rostock.de

Kiara Franke is a PhD student at the University of Rostock. She obtained a Master's degree in marine biology from the University of Bremen. During her Master's degree, she studied the temperature performance of kelps resulting in this manuscript. Her current main interest is on seasonal kelp primary production at a rocky shore site and how global warming is influencing kelp primary production. Her research is integrating physiology and biochemistry into ecological modelling.

Daniel Liesner
Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
Department of Algal Development and Evolution, Max Planck Institute for Developmental Biology, Max-Planck-Ring 5, 72076 Tübingen, Germany

Daniel Liesner is a postdoctoral fellow at Max Planck Institute for Developmental Biology in Tübingen, Germany. He is researching transitions in sexual systems of kelp, and is specialized in investigating trait variability and plasticity using ecophysiological and molecular methods.



Svenja Heesch
Applied Ecology and Phycology, University of Rostock, Albert-Einstein-Straße 21, 18059 Rostock, Germany

Svenja Heesch is a postdoctoral researcher at the University of Rostock in Germany. Her main interests are systematics and taxonomy, using genetic data in combination with morphology to unravel the biodiversity of marine as well as terrestrial macroalgae.

Inka Bartsch
Alfred Wegener Institute, Helmholtz Center for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
https://orcid.org/0000-0001-7609-2149

Inka Bartsch is a senior scientist at the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany. Apart from a wide interest in seaweed research and European Water Framework Directive monitoring, her major focus is on understanding the impact of abiotic and biotic factors on the regulation of kelp life cycle stages and communities, and their ability to cope with climate change. Having shifted from temperate NE-Atlantic locations towards the Arctic, she focusses on better resolving the inherent latitudinal complexity of kelp responses.