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Daylength influences reproductive success and sporophyte growth in the Arctic kelp species *Alaria esculenta*

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ABSTRACT: Many organisms have endogenous clocks that synchronize biological processes with environmental changes, leading to optimized development and reproduction. However, certain environments, like the Arctic, pose a special challenge to circadian clocks, particularly due to extreme seasonal changes in daylength, ranging from permanent sunlight to complete darkness. Kelps seem to be well adapted to the variable environmental conditions characteristic of this region. However, daylength might affect kelp species that use circadian rhythms to control the timing of daily egg release from female gametophytes. We aimed to investigate how daylength and light intensity affect gametogenesis and reproductive success of summer-reproducing kelp species (using *Alaria esculenta* as a model). As daylength and temperature co-vary most of the year, we also investigated the thermal resilience of the sporophytes developed under different daylengths to understand if there is a cross-tolerance between light doses and temperature tolerance. Although continuous daylight, characteristic of Arctic summers, enhanced gametogenesis and increased gametophyte vegetative growth, and thereby the number of potential reproductive gametophyte cells, sporophyte production was higher under long (16 h light:8 h dark) and intermediate (12:12 h) days. Sporophyte growth was triggered by changing daylength from short to long days, suggesting a synchronization with annual daylength variation. High daily light doses during reproduction and early development improved subsequent sporophyte survival at high (sub)lethal temperatures, indicating cross-tolerance between light and temperature. Reproductive success in Arctic *A. esculenta* was hampered under continuous light, and we hypothesize that this might result from disturbance of synchronized egg release and subsequent fertilization.

KEY WORDS: Biological rhythms · Brown alga · Gametogenesis · Photoperiod · Reproductive success

1. INTRODUCTION

The Arctic region is characterized by pronounced seasonal variations in daylength, light intensity and salinity, long periods of snow and ice cover and continuously low seawater temperatures (Wiencke et al. 2007). The polar regions pose a special challenge to the functioning of endogenous clocks — particularly due to the extreme seasonal changes in daylength, ranging from months of permanent sunlight to months of complete darkness (Häfker et al. 2018). The temporal coordination of internal clocks enables organisms to anticipate and adapt to daily and seasonal environmental changes by adjusting their

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physiological, behavioural and reproductive processes, thereby optimizing fitness (Suzuki & Johnson 2001, Dodd et al. 2005, Roenneberg & Merrow 2005, Emerson et al. 2008, Greenham & McClung 2015). However, there is a critical lack of knowledge on how the extreme photic conditions of the polar regions affect the endogenous rhythms of marine organisms, including large brown algae that form marine forests.

Kelp forests are important habitats along rocky coasts in Arctic regions, providing food, shelter and nursery for many marine organisms, including ecologically and commercially important species (Filbee-Dexter et al. 2019). Kelp species exhibit a heteromorphic life cycle, alternating between a microscopic haploid stage (gametophytes) and a macroscopic diploid one (sporophytes). Mature sporophytes release meiospores that germinate into male and female gametophytes. Sperm released from the mature male gametophyte fertilizes the egg, which develops into a sporophyte (Bartsch et al. 2008). In Laminariales, egg release from female gametophytes is controlled by a circadian rhythm, as was shown for 2 species (*Saccharina latissima*: Lüning 1981; *Undaria pinnatifida*: Zhang & Pang 2007). In *S. latissima*, after 8–10 d under long-day conditions (16 h light:8 h dark), eggs are released during the dark cycle (mainly during the first 30 min; Lüning 1981). This diurnal rhythm was maintained in the gametophytes transferred at the beginning of Day 8 from 16 h light:8 h dark to continuous darkness and in continuous red or green light, but not in continuous white or blue light. On the other hand, gametophytes exposed to continuous white light from Day 0 also started to release eggs after 8 d, but no rhythmic pattern was observed (Lüning 1981). Since continuous daylight can thereby suppress circadian egg release rhythmicity in *S. latissima*, this might be true for Laminariales in general. Thus, it is of utmost interest to investigate how continuous light affects reproductive success in summer-reproducing kelp species inhabiting the Arctic region.

The kelp *Alaria esculenta* (Linnaeus) Greville is a habitat-forming key species that populates sublittoral zones of the Arctic and cold temperate coastal ecosystems. In the North Atlantic, it occurs from the coast of Brittany (France) to the Arctic (Lüning 1990), with a fertile period throughout the summer during polar days in the Arctic and long days at lower latitudes (Olischläger & Wiencke 2013). As for some other North Atlantic kelp species, *A. esculenta* is predicted to expand its habitat range further into higher latitudes and greater annual daylength variation as Arctic ice recedes (Assis et al. 2018). Therefore, it is important to understand how these species tolerate extreme daylengths accompanied by additional environmental stressors. Organisms have evolved a variety of adaptive mechanisms to face the broad range of biotic and abiotic stresses to which they are exposed. Cross-talk or common elements between these mechanisms may result in cross-tolerance, where the exposure to an initial priming stressor elicits an improved response that protects the organism from a subsequent stressor of a different nature. There is growing evidence that cross-tolerance occurs in response to naturally co-occurring stressors (Rodgers & Isaza 2021).

Although priming is a well-established technique in terrestrial agriculture, it lags far behind in the aquaculture sector. Priming is now considered a promising strategy for the seaweed aquaculture sector, in which seaweeds acquire a stress memory that improves performance and resistance when exposed to a second stress and, thus, enhances production security at a low cost under environmental challenges (Jueterbock et al. 2021). Brown seaweeds of the genus *Alaria* are economically important species, widely used for a long time for direct consumption, nutritional supplements and cosmetics in many Asian countries and with growing interest in Europe (Kraan 2020). Therefore, the establishment of priming as a bio-engineering technique will help to move the aquaculture sector in Europe towards sustainable production.

How continuous light characteristic of Arctic summers affects the reproductive success and early sporophyte development — and therefore the persistence and expansion — of summer-reproducing kelps in polar environments remains an important unanswered question. To address this knowledge gap, we investigated the effect of different light conditions (photoperiod and irradiance level) on the gametogenesis and reproductive success of the Arctic kelp *A. esculenta*, which experiences continuous daylight for several months of the year and with the potential for northern range expansion under warming conditions. As photoperiod and temperature co-vary most of the year and influence recruitment and growth processes, the thermal resilience of the sporophytes raised under different daylengths was also investigated to understand if there is a cross-tolerance between light doses and heat tolerance which may provide a faster or stronger protective response against temperature through stress memory. Although several studies have explored the effect of photoperiod and temperature on gametogenesis, recruitment and sporophyte growth of *A. esculenta* (Makarov et al.

1999, Müller et al. 2008, Zacher et al. 2016), cross-tolerance between the 2 factors has not yet been investigated in this kelp species.

2. MATERIALS AND METHODS

2.1. Algal material

Four mature sporophytes of the kelp species *Alaria esculenta* were collected by divers in Kongsfjorden (Spitsbergen, Svalbard) in 2012. There, summer sea-surface temperatures are around 5–6°C (Hanelt et al. 2001). Sori were cleaned and zoospores from each individual were released separately into sterile seawater. After the development of gametophytes, female and male gametophyte stock cultures (AWI culture numbers — ♀: 3406, 3408, 3410, 3412; ♂: 3405, 3409, 3413, 3415) from each individual were established separately in Petri dishes (diameter: 8.9 cm; height: 3.5 cm) according to the protocol of Bartsch (2018). Vegetative gametophyte cultures were maintained in a climate-controlled chamber (Fitoclima, S600, Aralab) at 6°C under 3 µmol photons m^{-2} s^{-1} of red light, 16 h light:8 h dark (L:D) cycle in sterile full-strength Provasoli-enriched seawater (PES; Provasoli 1968) until the start of the experiment. The seawater was changed monthly.

2.2. Expt 1: Gametophyte growth, ontogenetic stages of gametogenesis and reproductive success

2.2.1. Experimental setup

The same amount of vegetative female and male gametophytes derived from the 4 *A. esculenta* individuals were mixed separately by sex and gently fragmented with a pestle and mortar. The 2 suspensions were sieved (diameter = 100 µm) and diluted in half-strength PES to produce a female and a male stock solution of gametophytes with lengths of ≤100 µm. Densities of each single-sex stock were calculated. Female and male gametophyte stock solutions were then combined, and the volume needed to achieve a gametophyte density of ~150 gametophytes cm^{-2} was added to Petri dishes (diameter: 5.3 cm; height: 1.5 cm) containing 12 ml of half-strength PES. Sterile artificial seawater (Tropic Marin sea salt) with a salinity of 30 psu was used.

The gametophytes were allowed to settle at 6°C under 3 µmol photons m^{-2} s^{-1} of red light for 1 d. After this period, the gametophytes were transferred to the target daylengths (24:0, 16:8, 12:12 and 8:16 h L:D cycles) and light intensities (10 and 20 µmol photons m^{-2} s^{-1} of white light) in a factorial design. Four replicate Petri dishes were used for each treatment (4 daylengths × 2 light intensities × 4 replicates = 32 Petri dishes in total). The temperature of 6°C was chosen to reflect the typical summer temperature of surface waters around Spitsbergen (Hanelt et al. 2001). The light intensities chosen were optimum to induce gametophyte reproduction in Laminariales (tom Dieck 1992, tom Dieck & de Oliveira 1993). In an attempt to disentangle the photoperiodic and dosage (i.e. total irradiance) effects of light, the same daily light dose of 0.864 mol photons m^{-2} d^{-1} was applied as 24 h of light d^{-1} with an intensity of 10 µmol photons m^{-2} s^{-1} and as 12 h light d^{-1} with 20 µmol photons m^{-2} s^{-1}. A daily light dose of 0.576 mol photons m^{-2} d^{-1} was also applied as 16 h light with an intensity of 10 µmol photons m^{-2} s^{-1} and 8 h light with 20 µmol photons m^{-2} s^{-1} (Table 1). Experiments were conducted in temperature-controlled climatic chambers (Fitoclima S600, Aralab). Irradiance was measured with a ULM-500 light meter (Walz). Culture medium was changed every week by the replacement of 6 ml of half-strength PES per Petri dish.

2.2.2. Gametophyte growth

To assess whether daylength and light intensity influenced gametophyte growth, the gametophyte area was measured, as it takes into consideration changes in length and diameter. Gametophyte area was quantified on Days 0 and 7 of each treatment by processing photographic data obtained from a Zeiss Axio Observer D1 inverted microscope (Carl Zeiss MicroImaging) with Q Imaging Retiga-SRV Fast 1394 camera (Q Imaging), using ImageJ software

Table 1. Light conditions used in the experiment on *Alaria esculenta*. Light conditions with the same daily light dose are marked with * and #

Daylength (light:dark)	Light intensity (µmol photons m^{-2} s^{-1})	Daily light dose (mol photons m^{-2} d^{-1})
24:0 h	10	0.864*
	20	1.728
16:8 h	10	0.576#
	20	1.152
12:12 h	10	0.432
	20	0.864*
8:16 h	10	0.288
	20	0.576#

(Schneider et al. 2012). The area of ≥18 female and male gametophytes was measured per replicate, corresponding to 10 randomly photographed fields of view (100× magnification). The gametophyte average area was determined per replicate dish. For fertile female gametophytes, oogonia were included, but eggs and developing sporophytes were excluded from area measurements.

Relative growth rates (RGRs) were estimated using the following formula: RGR (cm^2 cm^{-2} d^{-1}) = [ln(final area) − ln(initial area)]/T, where T is the culture period (days).

2.2.3. Quantification of ontogenetic stages

The relative occurrence of 3 ontogenetic stages of female gametophytes (1: vegetative state; 2: gametophytes with released eggs; 3: gametophytes with attached sporophytes) was quantified every 7 d over a period of 21 d in ≥300 female gametophytes per replicate using a Zeiss Axio Observer D1 inverted microscope. Gametophytes were considered to be in the egg-release or sporophyte stage if at least 1 cell per multicellular gametophyte had entered this developmental stage. Juvenile sporophytes were differentiated from released eggs if a first cell division was visible.

2.2.4. Reproductive success

Reproductive success was evaluated through the percentage of female gametophytes with sporophytes after 21 d, the absolute number of sporophytes with normal morphology per cm^2 after 29 d and the absolute number of sporophytes with irregular morphology per cm^2 after 29 d (i.e. possible partheno-sporophytes sensu tom Dieck 1992). Sporophytes with regular morphology (regular cell divisions, clear polar differentiation into basal rhizoid and proximal elongated blade) that remained attached to the respective female oogonium were considered to represent fertilized diploid sporophytes, while unattached sporophytes with non-polar irregular morphology and normally missing rhizoids were considered derived via parthenogenesis, a common feature of laminarialian and other brown algal life cycles (tom Dieck 1992, Oppliger et al. 2007, Bothwell et al. 2010). Most laminarialian partheno-sporophytes are generally unable to complete development and thus represent unsuccessful recruits (tom Dieck 1992). Although adult fertile partheno-sporophytes with normal morphology may develop in some species (*Undaria pinnatifida*: Shan et al. 2013; *Saccharina japonica*: Lewis et al. 1993), this does not usually occur in *A. esculenta* (Kraan et al. 2001). At this microscopic stage, we relied on morphological characteristics to discriminate between sporophytes and partheno-sporophytes as defined sensu tom Dieck (1992, Figs. 9–14), who fertilised female and mixed (female and male) gametophytes in separated cultures of several Laminariales and always observed partheno-sporophytes when eggs developed without males present and normal sporophytes when both sexes were present. DNA extraction and genetic analysis using sex-specific markers only proved possible after growth to a macroscopic stage. The percentage of female gametophytes with sporophytes was quantified in ≥300 female gametophytes per replicate, while the absolute number of sporophytes and partheno-sporophytes was evaluated in 100 fields of view (100× magnification) per replicate (Zeiss Axio Observer D1 inverted microscope).

2.3. Expt 2: Thermal tolerance of sporophytes

After 29 d (end of the reproductive experiment), the sporophytes developed under all daylengths at 20 µmol photons m^{-2} s^{-1} of white light were detached from the small Petri dishes (diameter: 5.3 cm; height: 1.5 cm), transferred to larger Petri dishes (diameter: 8.9 cm; height: 3.5 cm) and maintained under the same culture conditions. After 3 wk, sporophytes were transferred to sterile aerated 5 l glass bottles and kept in the same culture conditions for ~3 mo until sporophytes reached a length of 2–6 cm. Sterile PES was regularly changed 1–2 times per week. Sporophytes were acclimated for 1 wk to the daylength of 16:8 h L:D before the start of the experiment.

2.3.1. Discrimination of diploid and partheno-sporophytes

Sex-specific markers were used to investigate if the malformed sporophytes developed under continuous light were derived from sexual reproduction or unfertilised partheno-sporophytes, usually characterized by an irregular morphology. Only female marker amplification will be detected in unfertilised partheno-sporophytes, while normal diploid sporophytes will show amplification in both female and male sex markers. Thirty macroscopic sporophytes (2–6 cm length) developed under continuous light and 10 sporophytes

under the daylengths of 16:8, 12:12 and 8:16 h were randomly selected (including malformed and normal sporophytes) from the 5 l glass bottles before the start of the thermal experiment. Two female and 2 male gametophyte strains (♀: 3410, 3412; ♂: 3413, 3415) from *A. esculenta* were used to test and optimize the primers. Genomic DNA was extracted from silica-dried tissue using the Nucleospin 96 Plant II kit (Macherey-Nagel). The sex-specific markers were designed based on selected sex-specific sequences obtained by BLAST comparison of the *Ectocarpus* sp. sex chromosome sequences against *U. pinnatifida* (Lipinska et al. 2017). Primers were designed using Primer3Plus (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/). One female (UP-f_000170_1) and one male sex-specific marker (UP-13_001840_1) were optimized for *A. esculenta* (primer information in Table 2). PCRs were performed in a total volume of 20 µl containing 5 µl of 1:50 diluted DNA, 2.0 mM $MgCl_2$, 4.0 µl of 5× PCR buffer, 0.5 mM of dNTPs, 0.5 µM of forward and reverse primer and 1 U of GoTaq Polymerase (Promega). An initial denaturation step at 95°C for 5 min was followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s and a final extension step of 10 min at 72°C. The PCR products were separated by electrophoresis on 1.8% agarose gel and a 100 bp DNA ladder was used as a size reference.

2.3.2. Experimental temperature exposures

To understand if there is a cross-tolerance between light doses and heat tolerance in *A. esculenta*, the thermal resilience of the sporophytes raised under different daylengths was investigated. Five sporophytes (2–6 cm length) recruited and grown under each daylength (pre-daylength treatment) were transferred into each of the 4 replicated aerated beakers (1 l; half-strength PES) per target temperature. The temperature was slowly increased from 6°C to the target temperatures (18, 19, 20, 21 and 22°C, ±0.1°C) at a warming rate of 4°C d^{-1}, and sporophytes were exposed to each target temperature for 7 d. Temperatures between 18 and 22 °C were chosen to range the upper survival and lethal temperatures previously described for *A. esculenta* after 6 d of exposure (Fredersdorf et al. 2009). One set of beakers was left at 6°C (control). Culture medium was changed every 3 d. Experiments were conducted in temperature-controlled water-baths (Huber Variostat with Pilot ONE), provided with 20 µmol photons m^{-2} s^{-1} white LED light in a 16:8 h L:D regime. *A. esculenta* is a summer reproductive species, with spore germination, gametogenesis and sporophyte development possibly occurring from the beginning of summer to autumn (Olischläger & Wiencke 2013, Kraan 2020). We therefore focused on transitions from (1) summer to autumn, where decreases in photoperiod (continuous light to long days) and increases in the temperature can be observed, and (2) from spring to summer, where increases in photoperiod (short and intermediate days to long days) and temperature occur simultaneously. Transitions to and from the winter period that do not correspond to the reproductive season were not included. Tolerance to experimental temperatures was determined by quantifying relative growth rates and maximum quantum yield of photosystem II (PSII) (F_v/F_m) of sporophytes.

2.3.3. Relative growth rate

Sporophyte area was quantified on Days 0 and 7 by processing photographic data (Nikon D90 digital camera, Nikon Corporation) with ImageJ software (Schneider et al. 2012). RGR was estimated according to the formula used for gametophyte growth above.

2.3.4. Photosynthetic yield

F_v/F_m was measured on Day 7 in one random sporophyte from each of the 4 replicates per temperature and pre-daylength with a portable pulse amplitude modulated fluorometer (Diving-PAM; Waltz)

Table 2. Primer information for the sex markers in *Alaria esculenta*. NA: no amplification

Marker name	Primers		PCR product size (bp)	
	Forward	Reverse	Female	Male
UP-f_000170_1	CTT CTC GCT TTG TGG AGG GAA T	TAC GTG CGT CAT TCA GCA TTT G	456	NA
UP-13_001840_1	ACA TAG CTG AGG ATG GCG AAG C	CGA TGT CAG TGC CGT TAA GTG G	NA	492

and used as a proxy for physiological performance. Sporophytes were dark-acclimated for 5 min in leaf clip holders before the measurements. F_v/F_m is known to be a sensitive indicator of the photosynthetic performance in plants and seaweeds, with optimal values between 0.7 and 0.8 for brown algae (Büchel & Wilhelm 1993). Brown algae exposed to heat stress present values lower than this (Saada et al. 2016, Mota et al. 2018), which is closely related to the degree of photoinhibition (Papageorgiou & Govindjee 2004).

2.4. Statistics

2.4.1. Expt 1

Gametophyte relative growth rate, percentage gametophytes with sporophytes (arcsin square-root transformed), sporophyte density, partheno-sporophyte density (log transformed) and ratio of partheno-sporophytes/sporophytes (log transformed) data were evaluated by 2-factor ANOVAs (fixed factors: daylength, light intensity) using SPSS statistical package (v.23.0; SPSS). When significant interactions between treatments were observed, post hoc comparisons were performed (Tukey's multiple range test) to determine the responsible factor levels. Paired samples *t*-tests were performed for the treatments (daylength and light intensity) with the same daily light dose (0.576 and 0.864 mol photons m^{-2} d^{-1}) to disentangle the photoperiodic and dosage effects on gametophyte growth and sporophyte production and on sporophyte density data. To meet ANOVA assumptions, the data were checked for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test). Differences were considered significant at $p < 0.05$.

2.4.2. Expt 2

The relative growth data of sporophytes and the F_v/F_m data were analysed with PERMANOVA v.1.6 (Anderson 2001, McArdle & Anderson 2001), under a 2-factor design with pre-daylength and temperature as fixed factors. PERMDISP tests were performed to test the homogeneity of multivariate dispersions. Post hoc pairwise *t*-test comparisons were used to identify differences between treatments whenever a significant main effect or interaction was found. Analyses were performed with Euclidian distances and 9999 permutations. Differences were considered significant at $p < 0.05$.

3. RESULTS

3.1. Influence of daylength and light intensity on gametophyte growth, gametogenesis and reproductive success

RGRs of gametophytes differed significantly only due to daylengths (Table 3, Fig. 1); rates were 1.6-fold higher at continuous light (24:0 h) compared to 12:12 and 8:16 h.

The temporal development of ontogenetic stages under different daylengths is shown in Fig. 2. By Day 7, a small proportion of female gametophytes formed eggs (9–20%) under continuous light, while mostly only vegetative gametophytes were observed under L:D regimes (16:8, 12:12 and 8:16 h) in both light intensities. After 14 d of exposure, high proportions of female gametophytes became fertile, forming eggs

Table 3. Statistical analysis for the effects of daylength and light intensity on the relative growth rate of *Alaria esculenta* gametophytes after 7 d. Significant ($p < 0.05$) interactions or main effects are highlighted in **bold**. df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; *F*: *F*-statistic

Factor	df	SS	MS	*F*	p
Daylength	3	533.697	177.899	6.748	**0.002**
Light intensity	1	7.557	7.557	0.287	0.597
Daylength × light intensity	3	75.573	25.191	0.956	0.430

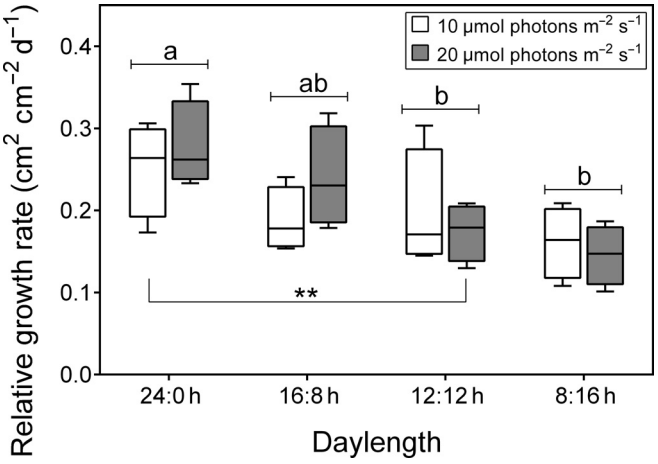

Fig. 1. Effects of daylength (24:0, 16:8, 12:12 and 8:16 h) and light intensity (10 and 20 µmol photons m^{-2} s^{-1}) on the relative growth rate of *Alaria esculenta* gametophytes after 7 d. Boxplots show median and 25th and 75th percentiles; whiskers: min. and max. values (n = 4). Different letters indicate differences among the means for each daylength ($p < 0.05$). Asterisks show a significant difference between 2 treatments of the same daily light dose (**$p < 0.01$). See Table 3 for statistics

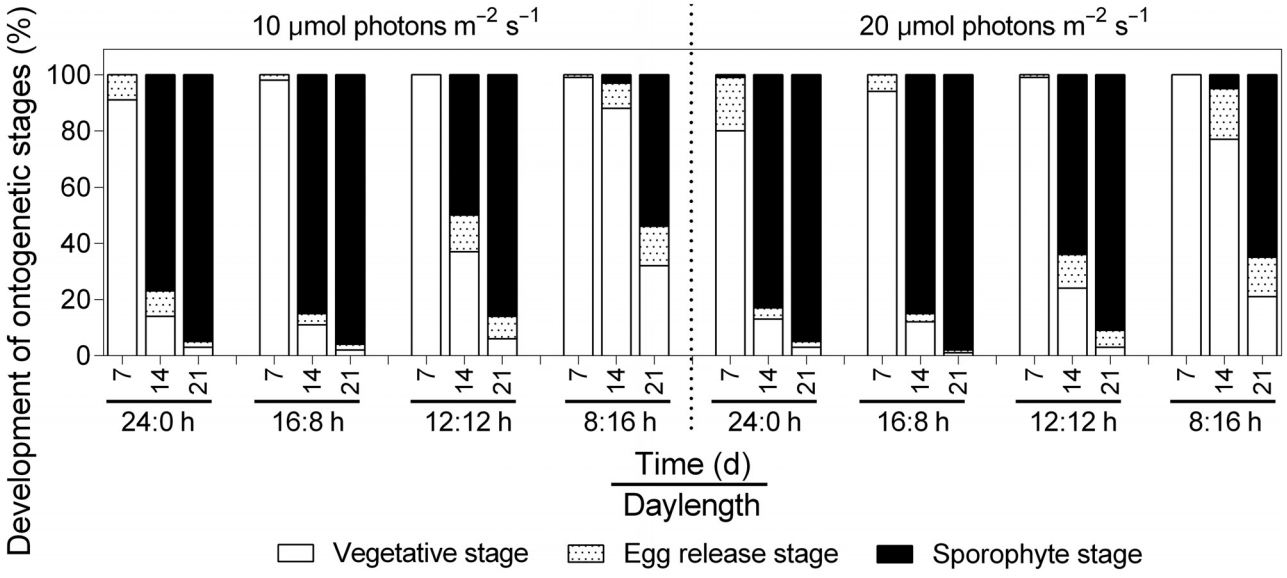

Fig. 2. Effects of daylength and light intensity on the development of ontogenetic stages of *Alaria esculenta* over time (7, 14, 21 d; mean values, n = 4). SE omitted for clarity

and sporophytes under continuous light (87%), 16:8 h (89%) and 12:12 h (70%) irrespective of the light intensity. In contrast, under short days (8:16 h) the development of fertility was delayed with only 18% of the female gametophytes fertile. On Day 21, almost all female gametophytes cultivated under the 3 longest daylengths (24:0, 16:8 and 12:12 h) developed a high proportion of sporophytes (86–98%), while the sporophyte formation was lower (54–65%) in the gametophytes exposed to the shortest daylength. In general, gametogenesis was faster under continuous light (24:0 h) and long days (16:8 h) than under 12:12 and 8:16 h.

The percentage of female gametophytes with sporophytes differed significantly with daylength and light intensity (Table 4, Fig. 3A), but there were no interactions between the 2 factors. The proportion of female gametophytes with sporophytes was negatively affected by a reduction in daylength, decreasing from a mean value of 96% at 24:0 and 16:8 h to 89% at 12:12 h and 59% at 8:16 h. In addition, relative sporophyte presence was significantly higher at 20 µmol photons m^{-2} s^{-1} (87%) than at 10 µmol photons m^{-2} s^{-1} (83%), irrespective of daylength. When the absolute number of sporophytes is considered, reproductive success showed a slightly different pattern (Fig. 3B). The sporophyte density differed significantly only due to daylengths (Table 4, Fig. 3B). The number of sporophytes developed was significantly higher under the daylengths of 16:8 and 12:12 h com-

Table 4. Effects of daylength and light intensity on the (a) percentage of female gametophytes with sporophytes, (b) absolute number of sporophytes per cm^2, (c) partheno-sporophytes per cm^2 and (d) partheno-sporophytes/sporophytes of *Alaria esculenta*. Significant ($p < 0.05$) interactions or main effects are highlighted in **bold**. df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; *F*: *F*-statistic

Factor	df	SS	MS	*F*	p
(a) Sporophytes (%)					
Daylength	3	1.289	0.430	121.702	**<0.001**
Light intensity	1	0.030	0.030	8.461	**0.008**
Daylength × light intensity	3	0.016	0.005	1.498	0.241
(b) Sporophytes (cm^{-2})					
Daylength	3	89 668.407	29 889.469	28.181	**<0.001**
Light intensity	1	55.879	55.879	0.053	0.820
Daylength × light intensity	3	2529.895	843.298	0.795	0.509
(c) Partheno-sporophytes (cm^{-2})					
Daylength	3	5.227	1.742	35.406	**<0.001**
Light intensity	1	0.895	0.895	18.198	**<0.001**
Daylength × light intensity	3	0.072	0.024	0.486	0.695
(d) Partheno-sporophytes/sporophytes					
Daylength	3	4.308	1.436	20.992	**<0.001**
Light intensity	1	0.749	0.749	10.953	**0.003**
Daylength × light intensity	3	0.002	0.001	0.011	0.998

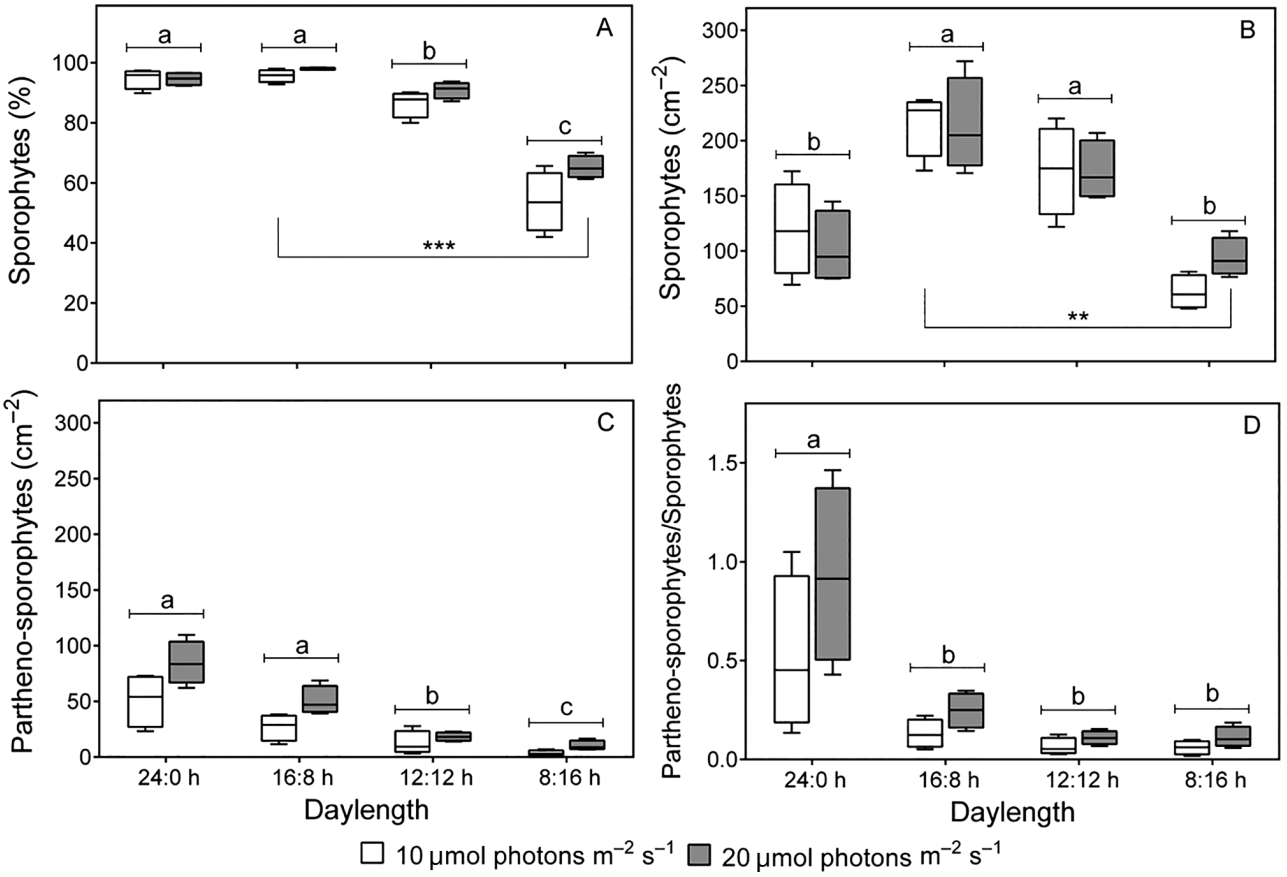

Fig. 3. Effects of daylength and light intensity on the reproductive success of juvenile sporophytes of *Alaria esculenta*. (A) Percentage of female gametophytes with juvenile sporophytes after 21 d. (B) Absolute number of sporophytes cm^{-2} after 29 d. (C) Absolute number of partheno-sporophytes cm^{-2} after 29 d. (D) Ratio of partheno-sporophytes number/sporophytes number after 29 d. Boxplots show median and 25th and 75th percentiles; whiskers: min. and max. values (n = 4). Different letters indicate differences among the means for each daylength ($p < 0.05$). Asterisks show significant differences between treatments of the same daily light dose (**$p < 0.01$; ***$p < 0.001$). See Table 4 for statistics

pared to 24:0 and 8:16 h. On the other hand, the number of malformed partheno-sporophytes and the ratio partheno-sporophytes number/sporophytes number differed significantly with daylength and light intensity (Table 4, Fig. 3C,D). In general, partheno-sporophytes developed in higher numbers at 20 µmol photons m^{-2} s^{-1} than at 10 µmol photons m^{-2} s^{-1} and under the 2 daylengths with more hours of light (24:0 and 16:8 h) compared to 12:12 and 8:16 h. The lowest number of partheno-sporophytes developed under the 8:16 h regime. Interestingly, the mean ratio partheno-sporophytes/sporophytes was much higher under continuous light (0.73) than in the L:D regimes (16:8 h = 0.19; 12:12 h = 0.09; 8:16 h = 0.09), indicating a reduction in egg fertilization by males in continuous light.

Significant differences were detected between treatments with the same daily light dose. RGR of gametophytes were higher ($p = 0.007$) at 24:0 h and 10 µmol photons m^{-2} s^{-1} than at 12:12 h and 20 µmol photons m^{-2} s^{-1} (Fig. 1), although they were exposed to the same daily light dose of 0.864 mol photons m^{-2} d^{-1}. The proportion of female gametophytes with sporophytes and the sporophyte density was higher ($p < 0.001$ and $p = 0.004$, respectively) under 16:8 h and 10 µmol photons m^{-2} s^{-1} than under 8:16 h and 20 µmol photons m^{-2} s^{-1} (Fig. 3A,B), both providing the daily light dose of 0.576 mol photons m^{-2} d^{-1}. However, no significant differences were detected in both reproductive success parameters and RGR of gametophytes between the treatments with the daily light dose of 0.864 and 0.576 mol photons m^{-2} d^{-1}, respectively.

3.2. Influence of daylength on sporophyte morphology

The macroscopic sporophytes developed at 6°C for 5 mo under the L:D regimes (16:8, 12:12 and 8:16 h)

were healthy and morphologically normal, while the sporophytes produced under continuous light (24:0 h) in general exhibited irregular morphology and light brown colour, particularly in the apical part (Fig. 4). Although malformed microscopic sporophytes may indicate parthenogenetic development from unfertilized eggs (tom Dieck 1992), the genomic DNA from all macroscopic sporophytes sampled per daylength showed amplification in both female and male sex markers (Fig. 5), demonstrating that they are diploids derived from sexual reproduction and carry both male and female haplotypes of the sex locus. No haploid partheno-sporophytes with only female marker amplification were detected in the sampled individuals after 5 mo of cultivation. As the macroscopic sporophyte sampling was performed after 5 mo of development, the microscopic partheno-sporophytes previously detected (Fig. 3C) may have already died, as they

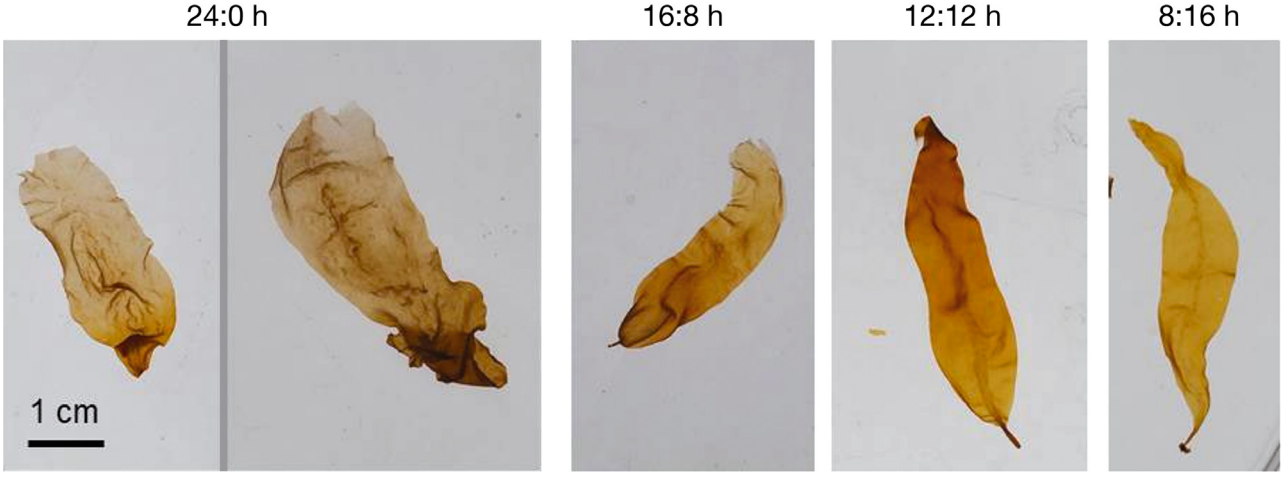

Fig. 4. Morphology of *Alaria esculenta* sporophytes developed under different daylengths. Two examples of sporophytes with irregular morphology developed under the 24:0 h daylength are shown. Sporophytes were grown at 6°C for ~5 mo. Scale bar = 1 cm (applies to all images)

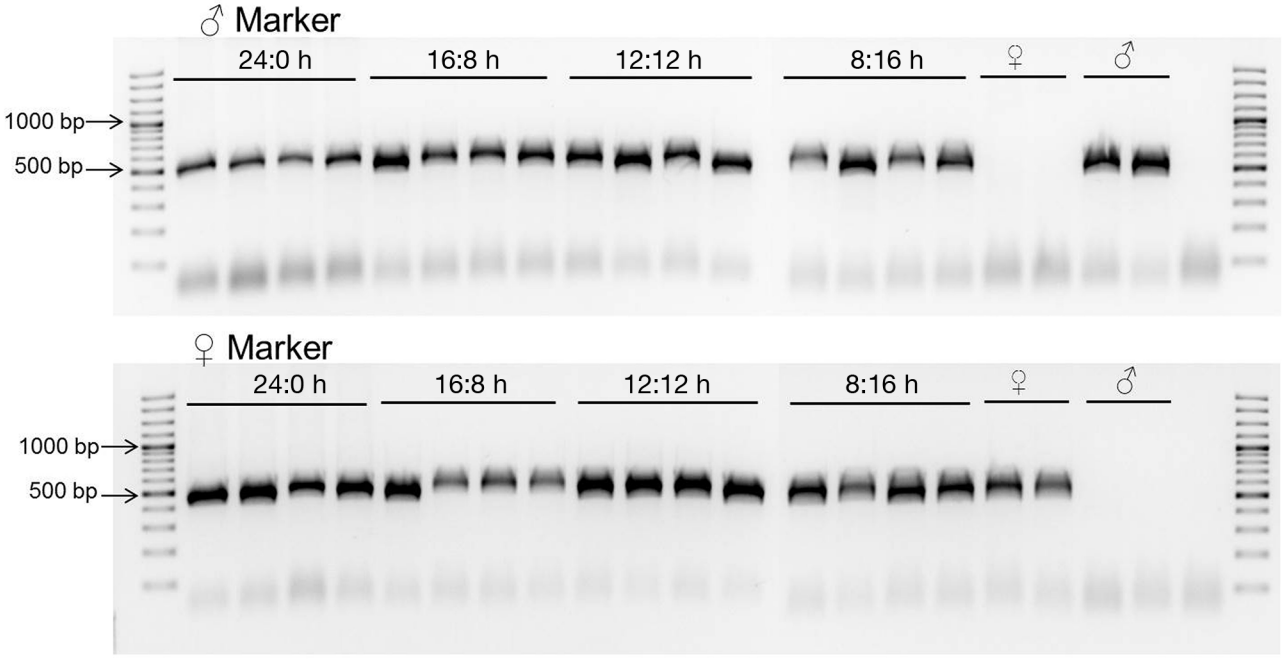

Fig. 5. Electrophoresis pattern of the sex-specific markers UP-f_000170_1 (female-specific) and UP-13_001840_1 (male-specific) products amplified in sporophytes developed under different daylengths and in female and male gametophytes of *Alaria esculenta*. Thirty sporophytes developed under continuous light and 10 developed under the light:dark regimes of 16:8, 12:12 and 8:16 h were used to amplify both sex-specific markers; however, only 4 sporophytes per treatment are shown as examples

have been reported to have high mortality rates, rarely reaching more than a few mm in length (tom Dieck 1992, Ar Gall et al. 1996, Druehl et al. 2005). Two female and male gametophytes of *Alaria esculenta* were used as controls, amplifying a product only for the female or male marker, respectively (Fig. 5).

3.3. Thermal tolerance of sporophytes

RGRs of sporophytes differed significantly due to the interaction between pre-daylength and temperature (Fig. 6, Table 5). Overall sporophyte RGR was negatively affected by temperature increase, but the temperature dependence of RGR (i.e. the negative slope of RGR) got steeper with decreasing pre-daylength light period (Fig. 6), suggesting that the pre-daylength treatment has somehow 'set' an intrinsic growth rate. Under control conditions (6°C), sporophyte RGR was strongly dependent on pre-daylength. Sporophytes which had been raised in continuous light (24:0 h) and long-day treatments (16:8 h) grew significantly slower than those developed in shorter daylengths (12:12 and 8:16 h) after transfer into the 16:8 h photoperiod used in this experiment (Fig. 6). Between 18 and 20°C, sporophytes coming from continuous light grew somewhat more slowly than those recruited under L:D cycles. Short-day sporophytes (8:16 h) showed negative growth rates at the highest temperature tested (22°C), which were significantly lower than those from sporophytes pre-cultivated in continuous and 16:8 h daylengths.

Table 5. Effects of temperature (6, 18, 19, 20, 21 and 22°C) on the relative growth rate of *Alaria esculenta* sporophytes previously developed under different daylengths. Significant ($p < 0.05$) interactions or main effects are highlighted in **bold**. df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; *F*: pseudo-*F* statistic

Factor	df	SS	MS	*F*	p(perm)
Pre-daylength	3	130.059	43.353	46.924	**<0.001**
Temperature	5	1113.609	222.722	241.066	**<0.001**
Pre-daylength × temperature	15	484.032	32.269	34.927	**<0.001**

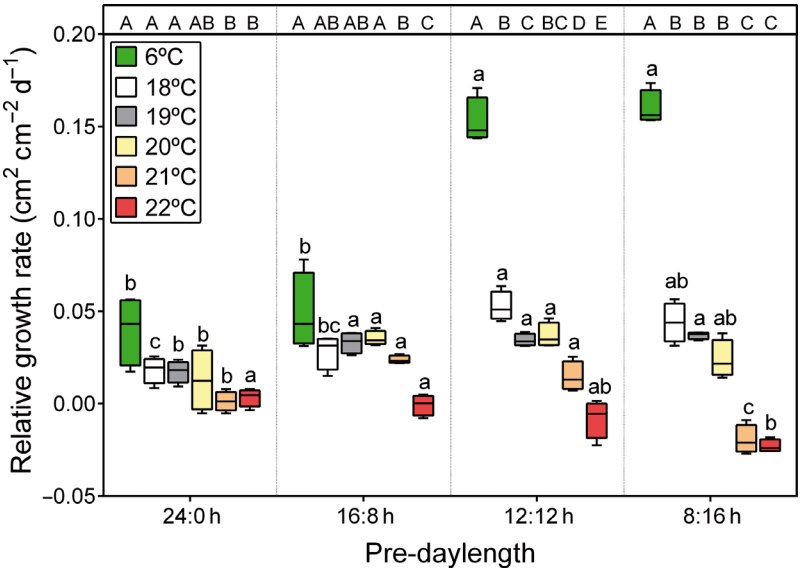

Fig. 6. Effect of temperature for 7 d on the relative growth rate based on area of *Alaria esculenta* sporophytes previously developed under different daylengths. Boxplots show median and 25th and 75th percentiles; whiskers: min. and max. values (n = 4). For each temperature, different lowercase letters indicate differences between daylengths ($p < 0.05$); for each daylength, different upper-case letters indicate differences between temperatures ($p < 0.05$). See Table 5 for statistics

F_v/F_m declined significantly with increasing temperature, but there were no significant effects of pre-daylengths or interactions between the factors (Table 6, Fig. 7). F_v/F_m values were highest at the control temperature of 6°C and at 18°C, with normal ratios of 0.7–0.8 for brown algae (Fig. 7). From 19°C onwards, the response of F_v/F_m suffered a progressive decline. At 20, 21 and 22°C, F_v/F_m significantly decreased to values of 0.48, 0.28 and 0.08, respectively, indicating severe photoinhibition or death, irrespective of the pre-daylength. The photosynthetic results were supported by the visual appearance of the sporophytes that presented some loss of pigmentation throughout the whole blade (more pronounced in the apical area) at temperatures ≥21°C after 7 d, irrespective of the daylength under which they previously developed.

4. DISCUSSION

This study provides new insights into the reproductive capacity of microscopic life stages of an Arctic population of the kelp species *Alaria esculenta* under different daylengths and light intensities (10 and 20 µmol photons m^{-2} s^{-1}). Exposure to continuous daylight, characteristic of Arctic summers, enhanced gametogenesis and increased gametophyte vegetative growth, however it suppressed reproductive success compared to long

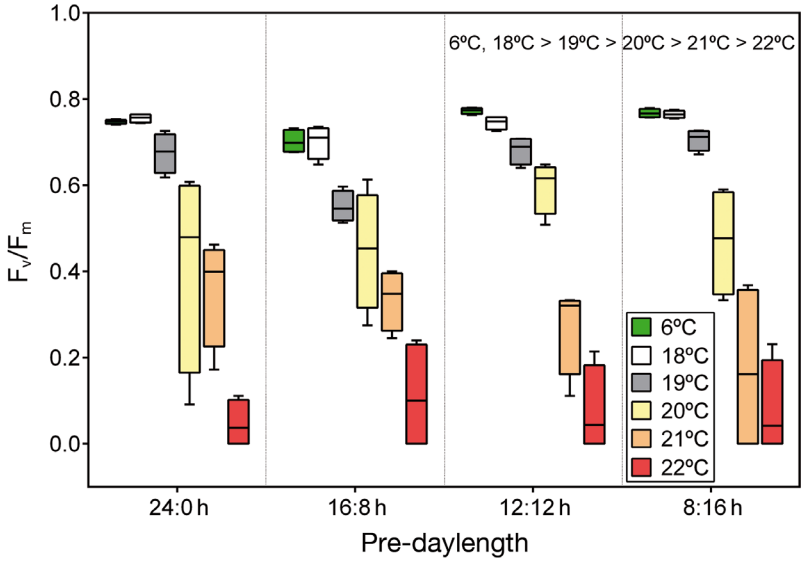

Fig. 7. Effect of temperature for 7 d on the maximum quantum yield of photosystem II (F_v/F_m) of *Alaria esculenta* sporophytes previously developed under different daylengths. Boxplots show median and 25th and 75th percentiles; whiskers: min. and max. values (n = 4). See Table 6 for statistics

Table 6. Effects of temperature (6, 18, 19, 20, 21 and 22°C) on the maximum quantum yield of PSII (F_v/F_m) of *Alaria esculenta* sporophytes previously developed under different daylengths. Significant ($p < 0.05$) interactions or main effects are highlighted in **bold**. df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; *F*: pseudo-*F* statistic

Factor	df	SS	MS	*F*	p(perm)
Pre-daylength	3	0.030	0.010	1.097	0.350
Temperature	5	5.892	1.178	129.861	**<0.001**
Pre-daylength × temperature	15	0.218	0.015	1.600	0.093

(16:8 h) and intermediate (12:12 h) days. The lower reproductive success under continuous light might be linked to a putative disturbance of synchronized egg release that supports coordinated fertilization by males at the onset of night due to the absence of blue light (Lüning 1981). This relationship between diurnal egg release and night has been shown in different members of the Laminariales (*Saccharina latissima* and *Undaria pinnatifida*) and may reflect a general pattern in this order. Our results also show that sporophyte growth is triggered by the daylength change from short to long days at the control temperature. In addition, it reveals that sporophyte growth under optimum temperatures and at upper thermal limits is highly dependent on the photoperiodic priming of the sporophytes during pre-cultivation.

4.1. Photoperiod controls gametophyte growth and reproductive success

Some studies indicate that the total light dose received by algae rather than the hours of illumination influences key biological processes (Chapman & Burrows 1970, Dring 1984), suggesting total irradiance as the primary factor controlling growth and reproduction. Lüning & Dring (1975) showed that the percentage of gametogenesis in *S. latissima* is proportional to the quantum dose of blue light received up to a saturating value of 400 µmol cm^{-2}. Additionally, threshold irradiance values below which seaweeds do not become reproductive are also reported in other seaweed species (*Callithamnion byssoides*: Kapraun 1978; *Desmarestia aculeata*: Chapman & Burrows 1970). In our study, higher reproductive success (percentage of female gametophytes with sporophytes and absolute number of sporophytes) was observed in the gametophytes under 16:8 h and 10 µmol photons m^{-2} s^{-1} than under 8:16 h and 20 µmol photons m^{-2} s^{-1}, although they were exposed to the same total irradiance dose of 0.576 mol photons m^{-2} d^{-1}. Additionally, significant differences in the growth rates of gametophytes were also detected at the total irradiance of 0.864 mol photons m^{-2} d^{-1} with higher growth rates in continuous light (24:0 h) and 10 µmol photons m^{-2} s^{-1} than in 12:12 h and 20 µmol photons m^{-2} s^{-1}. These results suggest the existence of a genuine photoperiodic effect that controls growth and reproduction in *A. esculenta* gametophytes. Final evidence for the existence of a true photoperiodic long day control would need additional experiments, including night break regimes in short-day treatments, as the duration of the night time is the controlling photoperiodic process (Vince-Prue 1986). Night break experiments have been used as a tool to study photoperiodic control of growth and development in plants and algae (Terry & Moss 1980, Lüning 1988, Pearce et al. 2017). In these experiments, the long night period is interrupted by a short white light period, thereby breaking up the night and converting a short day into a long day regime, triggering the development that normally only occurs during long daylengths (Vince-Prue 1986).

Several studies have shown that gametophyte growth in Laminariales is positively related to day-length, with larger gametophytes developing under long- than under short-days (*Laminaria digitata*: Martins et al. 2017; *Ecklonia radiata*: Mohring et al. 2013; *U. pinnatifida*: Choi et al. 2005; *Desmarestia ligulata*: Edwards 2000). In our study, *A. esculenta* gametophytes grew more under continuous light than under the daylengths of 12:12 and 8:16 h. *A. esculenta* gametogenesis was also promoted by continuous light and long-day conditions (16:8 h) and largely suppressed in short daylengths (8:16 h), particularly under low light intensities (10 µmol photons m^{-2} s^{-1}). A previous study also showed that *A. esculenta* fertility was optimized when gametophytes experienced a long exposure of light with at least 1 h of darkness (Duarte 2017). Similarly, gametogenesis was fastest under long days in *L. digitata*, which is also a summer-reproducing kelp species (Martins et al. 2017). The long day promotion of *A. esculenta* gametogenesis coincides with its seasonal reproduction pattern in the native environment, as spores successfully germinate between July and August (Olischläger & Wiencke 2013) when continuous light is present in polar regions and long days in the remaining distribution range of this species during the summer period.

4.2. Continuous light disturbs *A. esculenta* reproductive success

While at low latitudes the daily light–dark cycle enables robust entrainment of circadian clocks, organisms in high latitudes are faced with continuous daylight in summer and continuous darkness in winter, which can adversely affect the circadian system (Reierth et al. 1999, van Oort et al. 2005). In some members of the Laminariales, the circadian rhythmicity in egg release (Lüning 1981, Zhang & Pang 2007) together with the synchronized release of sperm driven by the pheromone lamoxirene (Lüning & Müller 1978, Müller et al. 1979) supports a highly coordinated process of egg fertilization by males. Since continuous light disturbs the circadian rhythmicity leading to the continuous release of eggs at a constant rate over a series of days in *S. latissima* (Lüning 1981), all the sperm are probably released during the first day(s) of egg extrusion, as male reproduction is protandric and the eggs released in the following days might fail to be fertilised, particularly as the life-span of sperm is generally no more than 12 h (Li et al. 2013). Our study showed that although *A. esculenta* exhibited high (female) gametogenesis rates under continuous light, the absolute number of sporophytes developed was much lower under this photoperiod compared to long (16:8 h) and intermediate (12:12 h) days. These results support that the daily egg release of *A. esculenta* is also controlled by the 'blue light off signal'. Lack of this signal might have hampered the coordinated fertilization of eggs by males and thereby reproductive success. This hypothesis is further supported by the high proportion of microscopic unfertilized partheno-sporophytes developed under continuous light.

4.3. Photoperiodism may constrain poleward *A. esculenta* expansion

Many temperate taxa are displaying poleward shifts in distributional range, as they track optimal thermal environments under climate warming (Huffeldt 2020). Since photoperiodic variation is particularly pronounced at the poles, with large variations over short latitudinal ranges, the northward expansion of organisms at high latitudes may be limited and/or disrupted by the adaptive capacity of the endogenous timing systems to such extreme photoperiods (Saikkonen et al. 2012, Huffeldt 2020). Most organisms have circadian rhythms that optimize fitness under the photic environment of their ancestral latitude. If the organisms are unable to adapt to extremes in photoperiod in the new high latitude range, the poleward range-shift of species will be restricted (Huffeldt 2020).

Predicting range expansions across latitudes requires a more comprehensive understanding of how species utilize light–dark cycles to coordinate seasonal growth, reproduction and synchronization of life cycles (Saikkonen et al. 2012). Although models have predicted that *A. esculenta* will successfully expand and persist into higher latitudes due to increasing temperatures (Assis et al. 2018), reproductive success might be hampered in these northern regions during polar days compared to more southern distribution areas characterized by light–dark cycles during summer. Therefore, our work indicates that photoperiod may constrain poleward *A. esculenta* expansion and emphasizes the importance of daylength as a key factor to consider for prediction of climate change-induced range expansion of species into higher latitudes.

4.4. Sporophytes raised under continuous light developed irregular morphology

During gametogenesis, a high proportion of microscopic partheno-sporophytes sensu tom Dieck

(1992) developed under continuous light. As parthenogenetic sporophytes of Laminariales have often been reported as having high mortality rates and rarely reaching more than a few mm in length (tom Dieck 1992, Ar Gall et al. 1996, Druehl et al. 2005), we wondered whether the macroscopic sporophytes developing in this condition but showing irregular morphology and less pigmentation were also of a possible parthenogenetic origin. Our genetic analysis confirmed that these macroscopic sporophytes were normal diploids resulting from sexual reproduction. The irregular morphology developed under continuous light might be attributed to the high level of blade morphological plasticity of kelp species driven by environmental factors (Demes et al. 2009, Coleman & Martone 2020, Coppin et al. 2020).

4.5. Cross-tolerance: high daily light doses during sporophyte formation improve the subsequent response to high (sub-)lethal temperatures

Most studies on kelp physiological responses to climate change have examined the effects of a single environmental variable, but in nature marine organisms are exposed to multiple environmental stressors (Wernberg et al. 2012). To generate more realistic inferences on the effects of global change, recent studies have focused on the interaction of multiple environmental stresses in kelps (Martins et al. 2017, Diehl et al. 2020, Fernández et al. 2021). However, organisms can also experience different stresses sequentially, and the exposure to a specific stressor may provide stress memory that enhances performance towards a subsequent different stressor, an effect known as cross-tolerance (Jueterbock et al. 2021, Yadav et al. 2021). Cross-tolerance has scarcely been considered in kelps (but see Springer et al. 2017). In our study, pre-cultivation at different daylengths influenced the RGR–temperature relationship after transfer to long-day conditions (16:8 h). Although the relationship between RGR and temperature shows in general a negative slope (i.e. RGR tends to decrease with increased temperature), the slope became steeper with decreasing pre-cultivation daylength. Therefore, exposure to high daily light doses improves the subsequent response to sublethal temperature stress. This ability for cross-tolerance might also be of relevance for *A. esculenta* populations or aquaculture at lower latitudes to improve sustainability during summer, particularly in southern sites.

4.6. Sporophyte growth follows the seasonal daylength cycle

Sporophyte growth in *A. esculenta* was substantially triggered by the daylength change from short to long days at the control temperature of 6°C, as the sporophytes pre-cultivated under shorter daylengths (8:16 and 12:12 h) showed 3.5-times higher growth rates after transfer to long days (16:8 h) compared to the sporophytes developed under long days and continuous light. Some *Laminaria* species are so-called season anticipators since their growth or reproduction is synchronized with the seasonal variation of daylength (Kain 1989). Previous experimental studies showed that *A. esculenta* sporophytes grew better under longer daylengths than in shorter days (Han & Kain 1996, Roleda et al. 2005), which is consistent with field observations. As with other kelp species, *A. esculenta* exhibits a phase of fast growth starting in early spring when the photoperiod increases rapidly and prevails until early summer followed by an autumn phase with reduced blade growth (Makarov et al. 1999). This pattern suggests that sporophytes of *A. esculenta* adjust their seasonal growth patterns in synchrony with annual changes in photoperiod. The higher growth rates of the short day pre-cultivated sporophytes were not due to higher daily light doses after transfer to long days as sporophytes kept under long days grew much less.

4.7. Upper thermal limits of *A. esculenta* sporophytes

Although no differences in photosynthetic efficiency were apparent in *A. esculenta* sporophytes pre-cultivated under different daylengths, photosynthesis was greatly influenced by the temperature treatments. *A. esculenta* sporophytes showed mean values of F_v/F_m close to maximum (0.70–0.77) at 6 and 18°C, which points to healthy and non-stressed tissue with an efficient conversion of light energy in PSII. Similar observations were made by Fredersdorf et al. (2009) for this species, where photosynthesis was unaffected by temperatures of ≤17°C for 6 d. On the other hand, exposure to temperatures of ≥20°C for 7 d induced slight bleaching of the blade and drastic decreases in F_v/F_m, indicating 20°C as the lethal temperature of sporophytes. However, the upper survival temperature of kelp species is dependent on stress exposure time. While sporophytes of *A. esculenta* die after 6–7 d at 20–21°C (Fredersdorf et al. 2009, this study), the limit decreases to

16–17°C after a few weeks (Munda & Lüning 1977). There remains the potential for inbreeding depression to affect thermal performance, as we used gametophytes from only 4 fertile sporophytes to produce the offspring sporophytes. However, the available evidence suggests this was not the case, as the upper thermal tolerance limit described here was similar to that from a previous study evaluating 14 field-collected sporophytes (Fredersdorf et al. 2009). In addition, as leaf morphology and anatomy can also affect photosynthetic capacity (Vemmos et al. 2013, Bhusal et al. 2018), certain F_v/F_m results need to be interpreted with caution, specifically for those sporophytes that developed under continuous light and which presented irregular morphology compared to the morphologically normal sporophytes grown under L:D regimes.

Our results show that although continuous light led to faster gametogenesis, it was less effective in the overall output of recruits compared to long and intermediate days. The lower reproductive success observed in the absence of an L:D cycle might be associated with the disturbance of synchronized egg release that supports the coordinated fertilization by males. Therefore, the predictions of successful expansion of *A. esculenta* northwards as sea ice recedes might be overestimated, as niche models do not take into consideration that species use L:D cycles to coordinate growth and reproduction. Sporophyte growth in *A. esculenta* was triggered by the daylength change from short to long days at 6°C, suggesting synchronization with the seasonal daylength cycle. High daily light doses during reproduction and early sporophyte development improves the subsequent sporophyte response to sublethal temperature stress, indicating cross-tolerance between light and temperature. Despite its summer fertility, the interaction of daylengths during reproduction and temperatures during early sporophyte growth suggests that the transition from cool short days to long-day conditions (especially spring to summer) will favour the early development of *A. esculenta*. Consequently, future warming might reduce the competitive strength of the species in southern areas above the Polar circle.

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LITERATURE CITED

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46

Ar Gall E (Le Gall Y), Asensi A, Marie D, Kloareg B (1996) Parthenogenesis and apospory in the Laminariales: a flow cytometry analysis. Eur J Phycol 31:369–380

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

Bartsch I (2018) Derivation of clonal stock cultures and hybridization of kelps. In: Charrier B, Wichard T, Reddy C (eds) Protocols for macroalgae research. CRC Press, Boca Raton, FL, p 61–78

Bartsch I, Wiencke C, Bischof K, Buchholz CM and others (2008) The genus *Laminaria* sensu lato: recent insight and development. Eur J Phycol 43:1–86

Bhusal N, Bhusal SJ, Yoon TM (2018) Comparisons of physiological and anatomical characteristics between two cultivars in bi-leader apple trees (*Malus* × *domestica* Borkh.). Sci Hortic (Amsterdam) 231:73–81

Bothwell JH, Marie D, Peters AF, Cock JM, Coelho SM (2010) Role of endoreduplication and apomeiosis during parthenogenetic reproduction in the model brown alga *Ectocarpus*. New Phytol 188:111–121

Büchel C, Wilhelm C (1993) *In vivo* analysis of slow chlorophyll fluorescence induction kinetics in algae: progress, problems and perspectives. Photochem Photobiol 58: 137–148

Chapman ARO, Burrows EM (1970) Experimental investigations into the controlling effects of light conditions on the development and growth of *Desmarestia aculeata* (L.) Lamour. Phycologia 9:103–108

Choi HG, Kim YS, Lee SJ, Park EJ, Nam KW (2005) Effects of daylength, irradiance and settlement density on the growth and reproduction of *Undaria pinnatifida* gametophytes. J Appl Phycol 17:423–430

Coleman LJM, Martone P (2020) Morphological plasticity in the kelp *Nereocystis luetkeana* (Phaeophyceae) is sensitive to the magnitude, direction, and location of mechanical loading. J Phycol 56:1414–1427

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

Demes KW, Graham MH, Suskiewicz TS (2009) Phenotypic plasticity reconciles incongruous molecular and morphological taxonomies: the giant kelp, *Macrocystis* (Laminariales, Phaeophyceae), is a monospecific genus. J Phycol 45:1266–1269

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

Dodd AN, Salathia N, Hall A, Kévei E and others (2005) Plant circadian clocks increase photosynthesis, growth, survival and competitive advantage. Science 309:630–633

Dring MJ (1984) Photoperiodism and phycology. Prog Phycol Res 8:159–162

Druehl LD, Collins JD, Lane CE, Saunders GW (2005) An evaluation of methods used to assess intergeneric hybridization in kelp using Pacific Laminariales (Phaeophyceae). J Phycol 41:250–262

Duarte A (2017) Optimization of seedling production using vegetative gametophytes of *Alaria esculenta*. MSc thesis, University of Porto

Edwards MS (2000) The role of alternate life-history stages of a marine macroalga: A seed bank analogue? Ecology 81:2404–2415

Emerson KJ, Bradshaw WE, Holzapfel CM (2008) Concordance of the circadian clock with the environment is necessary to maximize fitness in natural populations. Evolution 62:979–983

Fernández PA, Navarro JM, Camus C, Torres R, Buschmann AH (2021) Effect of environmental history on the habitat-forming kelp *Macrocystis pyrifera* responses to ocean acidification and warming: a physiological and molecular approach. Sci Rep 11:2510

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

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

Greenham K, McClung CR (2015) Integrating circadian dynamics with physiological processes in plants. Nat Rev Genet 16:598–610

Häfker NS, Teschke M, Hüppe L, Meyer B (2018) *Calanus finmarchicus* diel and seasonal rhythmicity in relation to endogenous timing under extreme polar photoperiods. Mar Ecol Prog Ser 603:79–92

Han T, Kain JM (1996) Effect of photon irradiance and photoperiod on young sporophytes of four species of the Laminariales. Eur J Phycol 31:233–240

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

Huffeldt NP (2020) Photic barriers to poleward range-shifts. Trends Ecol Evol 35:652–655

Jueterbock A, Minne AJP, Cock JM, Coleman MA and others (2021) Priming of marine macrophytes for enhanced restoration success and food security in future oceans. Front Mar Sci 8:658485

Kain (Jones) JM (1989) The seasons in the subtidal. Br Phycol J 24:203–215

Kapraun DF (1978) Field and cultures studies on growth and reproduction of *Callithamnion byssoides* (Rhodophyta, Ceramiales) in North Carolina. J Phycol 14:21–24

Kraan S (2020) Concise review of the genus *Alaria* Greville, 1830. J Appl Phycol 32:3543–3560

Kraan S, Rueness J, Guiry MD (2001) Are North Atlantic *Alaria esculenta* and *A. grandifolia* (Alariaceae, Phaeophyceae) conspecific? Eur J Phycol 36:35–42

Lewis RJ, Jiang BY, Neushul M, Fei XG (1993) Haploid parthenogenetic sporophytes of *Laminaria japonica* (Phaeophyceae). J Phycol 29:363–369

Li J, Pang S, Liu F, Shan T, Gao S (2013) Spermatozoid lifespan of two brown seaweeds, *Saccharina japonica* and *Undaria pinnatifida*, as measured by fertilization efficiency. Chin J Oceanology Limnol 31:774–781

Lipinska AP, Toda NRT, Heesch S, Peters AF, Cock JM, Coelho SM (2017) Multiple gene movements into and out of haploid sex chromosomes. Genome Biol 18:104

Lüning K (1981) Egg release in gametophytes of *Laminaria saccharina*: induction by darkness and inhibition by blue light and UV. Br Phycol J 16:379–393

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

Lüning K (1990) Seaweeds: their environment, biogeography and ecophysiology. John Wiley and Sons, New York, NY

Lüning K, Dring MJ (1975) Reproduction, growth and photosynthesis of *Laminaria saccharina* grown in blue and red light. Mar Biol 29:195–200

Lüning K, Müller DG (1978) Chemical interaction in sexual reproduction of several Laminariales (Phaeophyceae) release and attraction of spermatozoids. Z Pflanzenphysiol 89:333–341

Makarov VN, Makarov MV, Schoschina EV (1999) Seasonal dynamics of growth in the Barents Sea seaweeds: endogenous and exogenous regulation. Bot Mar 42: 43–49

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

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82:290–297

Mohring M, Wernberg T, Kendrick G, Rule M (2013) Reproductive synchrony in a habitat-forming kelp and its relationship with environmental conditions. Mar Biol 160: 119–126

Mota CF, Engelen AH, Serrão EA, Coelho MAG, Marbà N, Krause-Jensen D, Pearson GA (2018) Differentiation in fitness-related traits in response to elevated temperatures between leading and trailing edge populations of marine macrophytes. PLOS ONE 13:e0203666

Müller DG, Gassmann G, Lüning K (1979) Isolation of a spermatozoid-releasing and -attracting substance from female gametophytes of *Laminaria digitata*. Nature 279: 430–431

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

Munda IM, Lüning K (1977) Growth performance of *Alaria esculenta* off Helgoland. Helgol Wiss Meeresunters 29: 311–314

Olischläger M, Wiencke C (2013) Seasonal fertility and combined effects of temperature and UV-radiation on *Alaria esculenta* and *Laminaria digitata* (Phaeophyceae) from Spitsbergen. Polar Biol 36:1019–1029

Oppliger LV, Correa JA, Peters AF (2007) Parthenogenesis in the brown alga *Lessonia nigrescens* (Laminariales, Phaeophyceae) from central Chile. J Phycol 43:1295–1301

Papageorgiou GC, Govindjee A (2004) Chlorophyll *a* fluorescence: a signature of photosynthesis. Springer, Dordrecht, p 447–461

Pearce S, Shaw LM, Lin H, Cotter JD, Li C, Dubcovsky J (2017) Night-break experiments shed light on the photoperiod1-mediated flowering. Plant Physiol 174:1139–1150

Provasoli L (1968) Media and prospects for the cultivation of marine algae. In: Watanabe A, Hattori A (eds) Cultures and collections of algae. Proc US–Japan Conference, Hakone, 12–15 September 1966. Japanese Society of Plant Physiology, Tokyo, p 63–75

Reierth E, Van't Hof TJ, Stokkan KA (1999) Seasonal and daily variations in plasma melatonin in the High-Arctic Svalbard ptarmigan (*Lagopus mutus hyperboreus*). J Biol Rhythms 14:314–319

Rodgers EM, Isaza DFG (2021) Harnessing the potential of cross-protection stressor interactions for conservation: a review. Conserv Physiol 9:coab037

Roenneberg T, Merrow M (2005) Circadian clocks — the fall and rise of physiology. Nat Rev Mol Cell Biol 6: 965–971

Roleda MY, Hanelt D, Wiencke C (2005) Growth kinetics related to physiological parameters in young *Saccorhiza dermatodea* and *Alaria esculenta* sporophytes exposed to UV radiation. Polar Biol 28:539–549

Saada G, Nicastro KR, Jacinto R, McQuaid CD, Serrão EA, Pearson GA, Zardi GI (2016) Taking the heat: distinct vulnerability to thermal stress of central and threatened peripheral lineages of a marine macroalga. Divers Distrib 22:1060–1068

Saikkonen K, Taulavuori K, Hyvönen T, Gundel PE and others (2012) Climate change-driven species' range shifts filtered by photoperiodism. Nat Clim Chang 2:239–242

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9: 671–675

Shan TF, Pang SJ, Gao SQ (2013) Novel means for variety breeding and sporeling production in the brown seaweed *Undaria pinnatifida* (Phaeophyceae): crossing female gametophytes from parthenosporophytes with male gametophytes clones. Phycol Res 61:154–161

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

Suzuki L, Johnson CH (2001) Algae know the time of day: circadian and photoperiodic programs. J Phycol 37: 933–942

Terry LA, Moss BL (1980) The effect of photoperiod on receptacle initiation in *Ascophyllum nodosum* (L.) Le Jol. Br Phycol J 15:291–301

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

tom Dieck (Bartsch) I, de Oliveira EC (1993) The section Digitatae of the genus *Laminaria* (Phaeophyta) in the northern and southern Atlantic: crossing experiments and temperature responses. Mar Biol 115:151–160

van Oort BEH, Tyler NJC, Gerkema MP, Folkow L, Blix AS, Stokkan KA (2005) Circadian organization in reindeer. Nature 438:1095–1096

Vemmos SN, Petri E, Stournaras V (2013) Seasonal changes in photosynthetic activity and carbohydrate content in leaves and fruit of three fig cultivars (*Ficuscarica* L.). Sci Hortic (Amsterdam) 160:198–207

Vince-Prue D (1986) The duration of light and photoperiodic responses. In: Kendrick RE, Kronenberg GHM (eds) Photomorphogenesis in plants. Springer, Dordrecht, p 269–305

Wernberg T, Smale DA, Thomsen MS (2012) A decade of climate change experiments on marine organisms: procedures, patterns and problems. Glob Change Biol 18: 1491–1498

Wiencke C, Clayton MN, Gómez I, Iken K and others (2007) Life strategy, ecophysiology and ecology of seaweeds in polar waters. Rev Environ Sci Biotechnol 6:95–126

Yadav R, Juneja S, Kumar S (2021) Cross priming with drought improves heat-tolerance in chickpea (*Cicer arietinum* L.) by stimulating small heat shock proteins and antioxidative defense. Environ Sustain 4:171–182

Zacher K, Bernard M, Bartsch I, Wiencke C (2016) Survival of early life history stages of Arctic kelps (Kongsfjorden, Svalbard) under multifactorial global change scenarios. Polar Biol 39:2009–2020

Zhang Z, Pang S (2007) Circadian rhythms in the growth and reproduction of the brown alga *Undaria pinnatifida* and gametogenesis under different photoperiods. Acta Oceanol Sin 26:104–111

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