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Thermal priming of *Saccharina latissima*: a promising strategy to improve seaweed production and restoration in future climates

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ABSTRACT: *Saccharina latissima* is a brown algal kelp species of ecological and economic importance. As the rise in sea surface temperature will threaten not only wild populations of *S. latissima* but also the productivity of kelp farms, crop enhancement techniques will become crucial to mitigate this threat. Priming is a common strategy in crop plants, in which seeds are pre-exposed to moderate stress to improve the performance and tolerance of plants when exposed to harsher conditions. We investigated the potential of thermal priming to improve growth and tolerance of *S. latissima*. Kelp gametophytes primed at 20°C for 2, 4 and 6 wk and then re-transferred to 5°C were compared to a naïve treatment maintained at 5°C. Gametophyte priming increased growth of subsequently formed sporophytes by up to 30% (for 4 wk priming) compared to the naïve treatment. Female gametophyte growth in the priming environment was positively correlated to offspring sporophyte growth, indicating a maternal effect. Sporophytes were exposed to heat stress of 20°, 22°, 23° and 24°C for 2 wk. Sporophytes from 4 and 6 wk primed gametophytes exhibited 11 d longer tolerance at 22°C, 7 d longer tolerance at 23°C and 1°C higher thermal tolerance over 7 d compared to naïve sporophytes and sporophytes from 2 wk priming. A priming time of 4 wk was optimal for both sporophyte growth and thermal tolerance. Our results suggest that priming is a promising crop enhancement technique that could improve yield for seaweed farmers and restoration of kelp forests threatened by warming climates.

KEY WORDS: Kelp · Thermal priming · Stress memory · Crop enhancement techniques · Seaweed farming · Restoration · Thermal tolerance

1. INTRODUCTION

The seaweed industry is the fastest-growing aquaculture sector globally, with an annual expansion of 10% (Cai et al. 2021, Chopin & Tacon 2021). Seaweeds have been harvested for a wide array of applications (food products, animal feed, fertilizers, alginate and novel compounds; Bartsch et al. 2008), with an estimated value of US$ 14.85 billion in 2020 (Cai et al. 2021). While currently 95% of the global seaweed biomass is produced in Asian countries, production of seaweeds is rising in Europe (Cai et al. 2021, Chopin & Tacon 2021). In contrast with Asian countries, which cultivate most of their annual biomass (97%), Europe relies mainly on wild harvesting (99.5% of production; European Commission & Joint Research Centre 2019). Indeed, Norway, the leading European producer, has been harvesting 130 000–180 000 t of fresh weight annually of *Laminaria hyperborea* (Vea & Ask 2011) over the last 5 decades. However, wild har-

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vesting is destroying kelp forests, with detrimental effects on the associated biodiversity, ecosystem integrity and coastal erosion (Christie et al. 1998, 2003). Thus, an increase in biomass production must be reached by up-scaling seaweed cultivation, not by exerting additional pressure on wild kelp forests.

Saccharina latissima, also called sugar kelp, is the most cultivated species in Europe, representing 96% of the fresh seaweed biomass cultivated in 2015 (Stévant et al. 2017). *S. latissima* exhibits a haplo-diplontic heteromorphic life cycle, alternating between a macroscopic diploid sporophyte and microscopic haploid male and female gametophytes. *S. latissima* is a cold-temperate to Arctic species, widely distributed in the Northern Hemisphere in both the Atlantic and Pacific oceans (Neiva et al. 2018). Wild sporophyte tissue from Helgoland (Germany) was found to grow best between 10° and 15°C and survived 20°C over 2 wk, but not 23°C (Fortes & Lüning 1980, Lüning 1984). However, sporophytes from Brittany (France) that were collected in summer and slowly acclimated to heat stress were able to withstand 23°C for 8 d (Diehl et al. 2021). Nonetheless, this species does not generally occur at sites where summer temperatures exceed 20°C for an extended period (Diehl et al. 2021). Indeed, *S. latissima* is already decreasing in abundance in southern locations partly due to global warming (especially in the English Channel; de Bettignies et al. 2021). As marine heatwaves are expected to become more frequent and intense (Hobday et al. 2016, Oliver et al. 2018), *S. latissima* can be expected to suffer further habitat loss. In Norway, *S. latissima* forests have been classified as a highly threatened habitat on the Norwegian Red List for habitats (Choi et al. 2018). Warming waters will have concomitant effects on both wild populations and the production security of kelp farms. Thus, crop enhancement techniques are essential to mitigate the ecological and economic consequences of decreasing sugar kelp growth, performance and distribution.

Cultivars of the kelp species *S. japonica* and *Undaria pinnatifida* have been developed since the 1960s in Asia (Hu et al. 2021), but cultivar development of *S. latissima* including stress-resistant strains has only recently been initiated through various breeding projects (Ergon 2018, Lindell 2021, Umanzor et al. 2021, Li et al. 2022). However, one of the greatest challenges of seaweed breeding, besides the reduction of genetic variation, is the risk of cross-contamination between farmed and wild individuals (Shan et al. 2019, Hu et al. 2021), with the risk that only a few fast-growing genotypes will become dominant.

Priming, on the other hand, is a crop enhancement technique commonly used in agriculture (Liu et al. 2022) in which exposure of early life stages to a medium stress event improves performance and tolerance to later stress events. By exposing individuals to a priming environment, a 'stress memory' is triggered via epigenetic alterations that can persist across ontogeny, transferring desirable traits such as higher tolerance to the next generation (Wang et al. 2017, Mercé et al. 2020). Therefore, this process maintains genetic diversity and circumvents gene modification (Jueterbock et al. 2021). If thermal priming can enhance performance and stress tolerance in kelps, it could contribute to improving hatchery protocols for kelp farming to enhance sustainable production and restoration practices to enhance ecosystem recovery. In kelps, the first evidence of priming effects has been found in *Laminaria digitata*. By exposing gametophytes to 5°C for 1.5 yr, the growth of their offspring sporophytes at sublethal low and high temperatures (0° and 20°C) was improved compared to those grown at 15°C (Gauci et al. 2022), suggesting applications for kelp mariculture and/or restoration.

The main goal of our study was to test the effectiveness of a short-term priming protocol on *S. latissima* gametophytes that could be applied in a hatchery setup. We had 3 main questions: (1) Can warm priming of gametophytes change the growth of juvenile sporophytes? (2) Can warm priming of gametophytes improve the upper-temperature survival of offspring sporophytes exposed to a heat-stress scenario? (3) How long must a priming signal on gametophytes act to trigger an effect in offspring kelp sporophytes?

2. MATERIALS AND METHODS

To identify the impact of priming duration on the growth and tolerance of *Saccharina latissima*, we ran a common garden experiment (Fig. 1). Gametophytes that were pre-cultivated at 5°C were primed for 2, 4 and 6 wk at 20°C, the mean summer sea surface temperature at the species' southernmost distribution (Diehl et al. 2021). Following priming, samples were directly transferred back to 5°C for gametogenesis. These were compared to a naïve treatment (5°C) that was not primed and went straight into gametogenesis. Gametogenesis of naïve and primed gametophytes was induced at 5°C and ontogenetic stages were quantified for 2 wk. Sporophyte growth was measured for 1 wk after gametogenesis under the same conditions to detect any performance change associated with priming. Then, 2 wk old microscopic sporo-

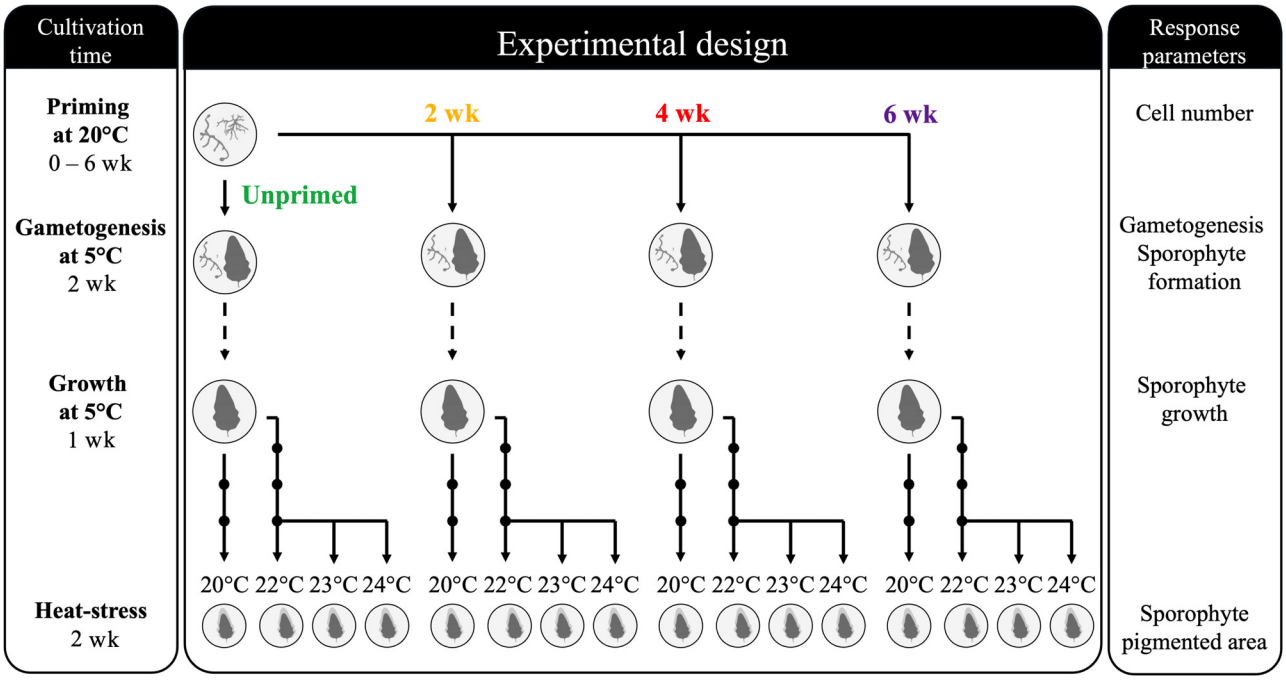

Fig. 1. Common garden experiment to investigate the effects of temperature priming duration on gametogenesis of *Saccharina latissima* gametophytes, and growth and thermal tolerance of the juvenile offspring sporophytes (n = 5 per treatment)

phytes were transferred to a heat-stress gradient, where the temperature was increased by 5°C every 3 d until reaching 20°, 22°, 23° or 24°C, during which the pigmented area was measured every 3–4 d for 2 wk as a proxy for survival to detect changes in the thermal tolerance of the species via priming.

2.1. Preparation of experimental material and priming

Clonal isolates of 6 pairs of male and female gametophytes derived from separate sporophytes of *S. latissima* sampled from Oslo (1 pair), Bergen (1 pair) and Spitsbergen (4 pairs) were used (Table A1 in the Appendix). Prior to the start of the experiment, the gametophytes were maintained at 5°C under an irradiance of 2 µmol photons m^{-2} s^{-1} red light, in a 16 h light:8 h dark cycle (Mitras Lightbar 2 Daylight, controlled by a Profilux 3 computer; GHL Advanced Technology), in 50% Provasoli-enriched seawater (PES; Provasoli 1968) without iron to prevent gametogenesis (Lüning & Dring 1975, Lewis et al. 2013).

To follow gametogenesis and sporophyte formation, male and female stock solutions were prepared separately by pooling similar amounts of each male or female clonal vegetative gametophyte culture (Bartsch 2018). Gametophytes were gently crushed in a mortar and filtered through a 100 µm sieve. Following that, 1 ml was inoculated in a plastic dish (diameter: 6 cm, Coria, polystyrol, VKF Renzel) containing 50 ml of 50% PES. After 15 min of settlement, the number of multi-cellular vegetative gametophytes in 40 fields of view was counted using an inverted microscope (CKX41, Olympus) at 100× magnification with a 1 mm^2 ocular grid. The inoculate volume was then adjusted to reach a target density of approximately 300 gametophytes cm^{-2} per sex. A total of 20 dishes were inoculated per priming treatment to ensure n = 5 replicates per temperature for the final heat-stress experiment.

Priming was performed in Huber Variostat water baths with a Pilot ONE (Peter Huber Kältemaschinenbau) by increasing the temperature by 5°C every 3 d until reaching 20°C. Gametophytes were primed for 2, 4 and 6 wk at 20°C under an irradiance of 0.5 µmol photons m^{-2} s^{-1} white light.

2.2. Gametogenesis

Following priming, gametophytes were directly transferred from 20°C to 5°C without acclimation to ensure the last thermal experience was the priming

temperature. Gametogenesis was induced by increasing the irradiance by 5 µmol photons m^{-2} s^{-1} white light per day until reaching 15 µmol photons m^{-2} s^{-1} white light. Iron-free PES medium was replaced by 50% PES at this stage.

Gametophyte density and 4 ontogenetic stages of gametogenesis — vegetative gametophytes, gametophytes bearing oogonia, gametophytes with eggs released and gametophytes with offspring sporophytes attached (Martins et al. 2017) — were quantified on Day 0, 3, 7, 10 and 14 following the same subset of replicates over time (n = 5 per priming duration). The number of cells per multi-cellular female gametophyte was counted on Day 0 to quantify the female gametophyte growth and the potential number of sporophytes that could be produced, as each female cell may produce a single egg (Bolton & Lüning 1982). For each multi-cellular female gametophyte, the most advanced stage of development was noted, i.e. when at least one of the gametophyte cells had entered the respective stage of development. Absolute numbers of recruited sporophytes were counted on Day 14. The medium in the Petri dishes was changed after 14 d.

2.3. Sporophyte growth

Sporophytes were photographed after 14 and 21 d at 5°C under an inverted microscope. In total, 10 pictures were taken per replicate and the length of the largest sporophyte per field was measured using ZEN software (Carl Zeiss). As sporophytes born at different time points during gametogenesis following each priming treatment, they had a different initial size. Therefore, the relative growth rates (RGR) were calculated over 1 wk using the formula (Franke et al. 2021):

$$\mathrm{RGR}\,(\%\ \mathrm{week}^{-1}) = \frac{\frac{x_f - x_i}{x_i}}{t_f - t_i} \times 100 \quad (1)$$

where x_f is the sporophyte length (in µm) at the final time, x_i is the length at the initial time, t_f is the final time (in weeks) and t_i is the initial time.

We predicted the length of sporophytes over time for each priming duration and the naïve treatment for the first 6 wk, which is the recommended time in hatchery (Forbord et al. 2020), assuming a constant growth rate, using the formula:

$$x_t = x_i \times (1 + \mathrm{RGR})^t \quad (2)$$

where x_t is the expected sporophyte length (in m) at time t, x_i is the sporophyte length at the initial time (in m), RGR is the relative growth rate calculated above (in % wk^{-1}) and t is time at week t.

2.4. Sporophyte thermal tolerance

After 21 d at 5°C, the tolerance of sporophytes resulting from gametophytes with different thermal priming durations was tested under heat stress (20, 22, 23 and 24°C) for 2 wk (n = 5). The temperatures were increased by 5°C every 3 d until reaching 20°C, and raised over 3 more days to reach the final temperatures (22, 23 and 24°C). Replicates for the 20°C heat stress started the acclimation 3 d later to ensure that all dishes reached the final temperature on the same day. The medium in the Petri dishes was changed at the start of the heat stress.

Five pictures per replicate were taken to ensure coverage of the dish area on Day 0, 3, 7, 10 and 14 of the heat stress, using a stereo microscope at 10× magnification (SZX10, Olympus). Pictures were processed individually using Fiji (Schindelin et al. 2012). The sporophyte canopy was separated from the background using a color threshold (Fig. 2). By adjusting the hue to range from 0 to 70, saturation from 40 to 255 and brightness from 0 to 255, we could measure the total area of the canopy. Then, by reducing the hue to range from 0 to 40, we separated the pigmented area of the canopy (healthy) from the bleached area and measured it. The measured areas from the 5 pictures of the same replicate were summed and adjusted to 1 cm^2. Initial bleaching of thalli may occur over all parts of the blade, but after some time it appeared that the meristematic area stayed pigmented the longest (Fig. A1, C. Gauci unpubl. data); thus, we used the pigmented area as a proxy for survival. We defined survival as >5% of pigmented area, as only the stipes and no living thallus area of sporophytes could be clearly identified below this threshold.

2.5. Statistical analysis

All analyses were performed in R v.4.0.3 (R Core Team 2020). Normality of residuals was verified with a Shapiro test, and homoscedasticity between treatments was assessed with Levene's test. The number of cells produced per female gametophyte in the priming environment was compared between priming durations using 1-way ANOVA. The densities of female gametophytes bearing eggs and sporophytes were summed to estimate the reproductive success after 14 d at 5°C. The effect of different priming dura-

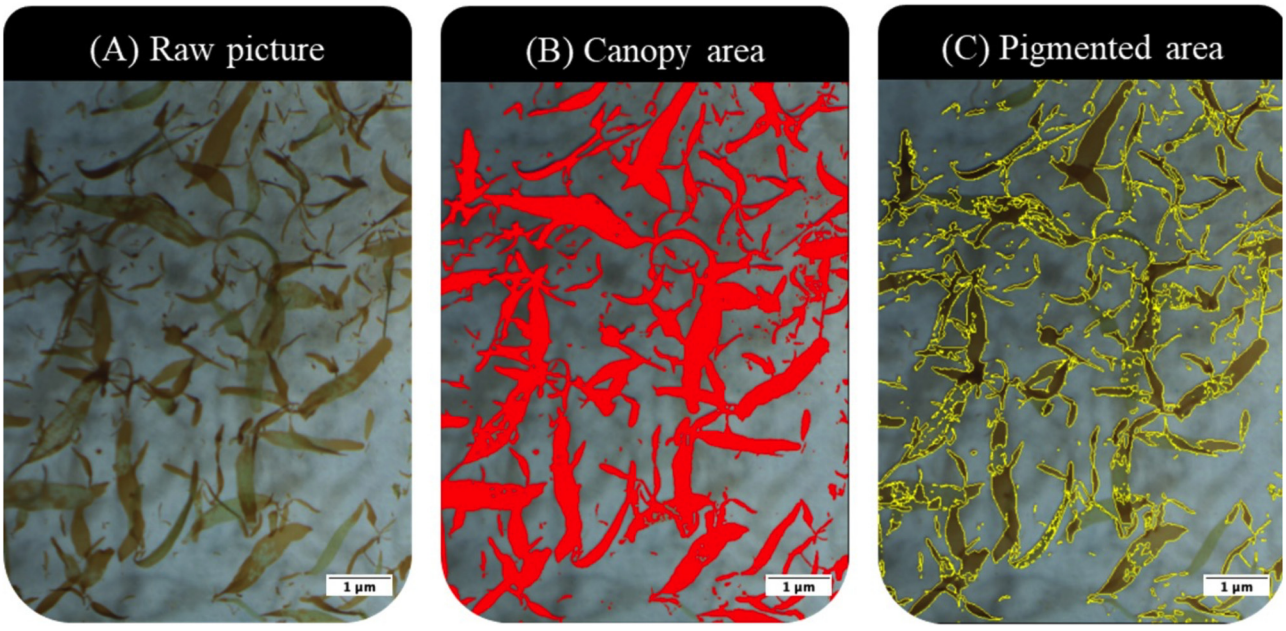

Fig. 2. Example of the (A) raw image and the 2-color thresholds performed with ImageJ to measure (B) the canopy area and to discriminate (C) the healthy pigmented area of *Saccharina latissima* sporophytes during heat stress as a proxy for survival. As a result, the area of (B) was always greater than (C) unless no bleaching occurred

tions at 20°C on this measure of reproductive success at Day 14 was analyzed using 1-way ANOVA.

As gametophytes grew over a differential period during priming (2, 4, 6 wk), this resulted in different numbers of female cells at the start of the experiment. Consequently, the sporophyte density was normalized to the number of cells per female. The coefficient of normalization was calculated by dividing the number of cells per female for each replicate of each priming duration by the overall mean of cells per female. In the end, sporophyte densities were multiplied by the coefficient of normalization, and the effect of priming duration on the sporophyte density was investigated using 1-way ANOVA.

The effect of priming duration on sporophyte growth and the predicted sporophyte length after 6 wk was analyzed using 1-way ANOVA.

The relationship between the female gametophyte proliferation during priming (in cell number) with the RGR of the offspring sporophytes (in % wk^{-1}) was analyzed with a Pearson correlation analysis including the naïve gametophytes and their offspring sporophytes.

Because some replicates started bleaching during the acclimation, the pigmented area was significantly different between priming durations and was therefore normalized to Day 0 for each replicate and expressed as a percentage. We evaluated the percentage of pigmented area of sporophytes over time in a heat-stress gradient after differential priming duration of the parental gametophytes with 2-way repeated-measures ANOVA (corrected with Mauchly's sphericity test) for each temperature. The effect of priming duration on the sporophyte pigmented area on Day 14 was analyzed using 1-way ANOVA. The time that led to a loss of 50% of the pigmented area (LT50) was determined using a linear quadratic model for survival using GraphPad Prism v.10.0.0 (GraphPad Software). Models were built for each heat-stress temperature except for 20°C because sporophytes from all priming durations exhibited more than 50% remaining pigmented area.

3. RESULTS

3.1. Priming and gametogenesis

The number of cells per female gametophyte significantly increased with priming duration (1-way ANOVA, $F_{3,16} = 48.93$, $p < 0.001$) until a maximum was reached after 4 wk (Fig. 3).

The speed of gametogenesis was significantly affected by priming duration (1-way ANOVA, $F_{3,16} = 13.466$, $p < 0.001$). Naïve gametophytes and gametophytes primed for 2 wk at 20°C completed gametogenesis more rapidly at 5°C than gametophytes primed for 4 and 6 wk (Fig. 4). First sporophytes

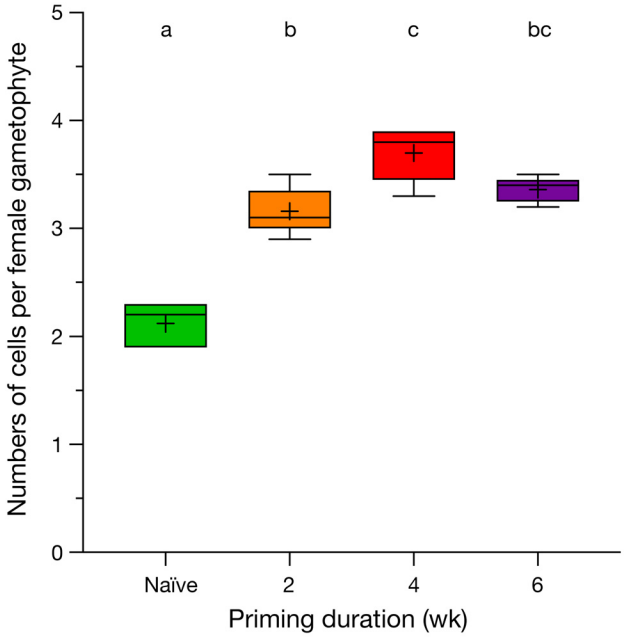

Fig. 3. Absolute number of female *Saccharina latissima* gametophyte cells of naïve and primed gametophytes after 2, 4 or 6 wk of priming at 20°C (mean ± SE, n = 5). Boxplots represent the median as a line; cross as the mean number of cells per female gametophytes; interquartile range as a box; minimum and maximum values as whiskers. Letters represent significant differences between priming duration ($p < 0.01$, Tukey tests)

appeared after 7 d for naïve gametophytes and those primed for 2 wk but only after 10 d for gametophytes primed for 4 and 6 wk. Gametophytes primed for 2 wk not only exhibited the fastest gametogenesis but also the most successful gametogenesis, with 83% of female gametophytes releasing eggs (2 wk > naïve = 4 wk = 6 wk; Tukey tests, $p < 0.021$ for all). Although naïve gametophytes exhibited faster gametogenesis compared to gametophytes primed for 4 and 6 wk, reproductive success was not significantly different between the 3 (74, 66 and 66% of female gametophytes had respectively undergone full development; Tukey tests, $p > 0.05$ for all).

Gametophyte densities on Day 0 were not significantly different between priming durations (1-way ANOVA, $F_{3,16} = 1.182$, $p = 0.348$; overall mean: 1107 ± 89 gametophytes cm^{-2}), and neither was the sex ratio on Day 3 (1-way ANOVA, $F_{3,16} = 0.399$, $p = 0.755$; overall mean: 1.8:1 male:female ratio). Therefore, only the number of cells per female gametophyte, i.e. the number of eggs potentially produced, could explain differences in sporophyte formation.

The faster gametogenesis of gametophytes primed for 2 wk resulted in a significantly higher normalized sporophyte density after 14 d of gametogenesis compared to all other priming durations (1-way ANOVA, $F_{3,16} = 12.116$, $p < 0.001$ for all tests, Tukey tests).

3.2. Sporophyte growth

The sporophyte RGR was significantly affected by the priming duration of their parental gametophytes (1-way ANOVA, $F_{3,16} = 10.556$, $p < 0.001$; Fig. 5A). The fastest-growing sporophytes were derived from gametophytes primed for 4 wk, as they grew 30% faster than the naïve ones (Tukey tests, $p < 0.001$). Sporophytes derived from gametophytes primed for 2 wk grew at a similar rate to those from naïve gametophytes or those primed for 6 wk.

Predicted sporophyte length was significantly affected by the priming duration of their parental gametophytes (1-way ANOVA, $F_{3,16} = 8.187$, $p = 0.002$). Sporophytes derived from gametophytes primed for 4 wk are predicted to be 2.5× longer than naïve ones (Tukey tests, $p = 0.001$; Fig. 5B). Offspring sporophyte growth was significantly positively correlated with the growth of their parental female gametophytes (Pearson test, $R = 0.65$, $p = 0.0019$; Fig. 6).

3.3. Sporophyte thermal tolerance

At 20°C, the pigmented area as a proxy for survival was not significantly affected by priming (Table 1). However, the pigmented area at 22°, 23° and 24°C was significantly enhanced by priming duration and time in the heat stress (Table 1). At 22°C, the pigmented area of sporophytes from 4 and 6 wk priming never dropped below 50% (Fig. 7A) and was significantly higher than the pigmented area of naïve sporophytes after 14 d (Tukey tests, $p < 0.009$ for all tests; Fig. 8).

At 23°C, naïve sporophytes and sporophytes from 2 wk priming reached the 5% threshold (which we considered detrimental) after 14 d, unlike sporophytes from 4 and 6 wk priming, which exhibited 12 and 35% pigmented areas, respectively (Fig. 7B). Sporophytes from 6 wk priming exhibited the longest LT50 ($LT50_{6\,wk} = 8.2$ d), followed by those primed for 4 wk ($LT50_{4\,wk} = 6$ d) and those primed for 2 wk ($LT50_{2\,wk} = 4$ d), while the naïve sporophytes lost 50% of pigmented area after only 2.7 d at 23°C.

At 24°C, the pigmented area of sporophytes from 4 wk priming after 7 d was significantly higher (33%) than the pigmented area of sporophytes from the naïve ones and from 2 and 6 wk priming, which fell below the 5% threshold (1-way ANOVA,

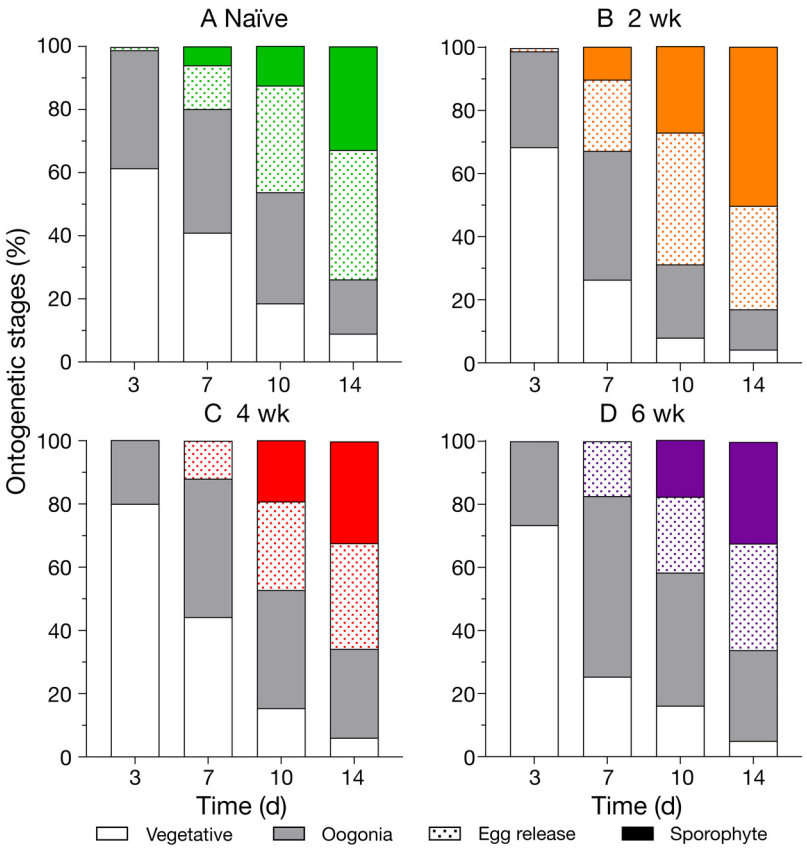

Fig. 4. Development of ontogenetic stages of female *Saccharina latissima* gametophytes over time at 5°C for (A) naïve cells, and after (B) 2 wk, (C) 4 wk and (D) 6 wk of priming at 20°C (mean values, SE omitted for clarity; n = 5)

$F_{3,15}$ = 23.734, p < 0.001 for all tests, Tukey tests; Fig. 7C). Furthermore, the LT50 was reached 2 d later for the sporophytes from 4 wk priming compared to other priming durations ($LT50_{4\,wk}$ = 4.6 d > $LT50_{Naïve\,=\,2\,wk\,=\,6\,wk}$ = 2 d).

At 22°C, the pigmented area of sporophytes from 4 and 6 wk priming after 14 d was not significantly different than the pigmented area of sporophytes from the naïve and 2 wk priming after only 3 d, indicating 11 d longer tolerance (1-way ANOVA, $F_{3,16}$ = 0.262, p = 0.851). At 23°C, the pigmented area of sporophytes from 4 and 6 wk priming after 14 d was not significantly different than the pigmented area of sporophytes from the naïve and 2 wk priming after 7 d, indicating 7 d longer tolerance (1-way ANOVA, $F_{3,16}$ = 1.507, p = 0.251). At 24°C, the pigmented area of sporophytes from 4 wk priming after 7 d was not significantly different than the pigmented area of sporophytes from other priming durations after 3 d, indicating 4 d longer tolerance (1-way ANOVA, $F_{3,15}$ = 0.151, p = 0.927).

The pigmented area of sporophytes from 4 wk priming after 7 d at 24°C was

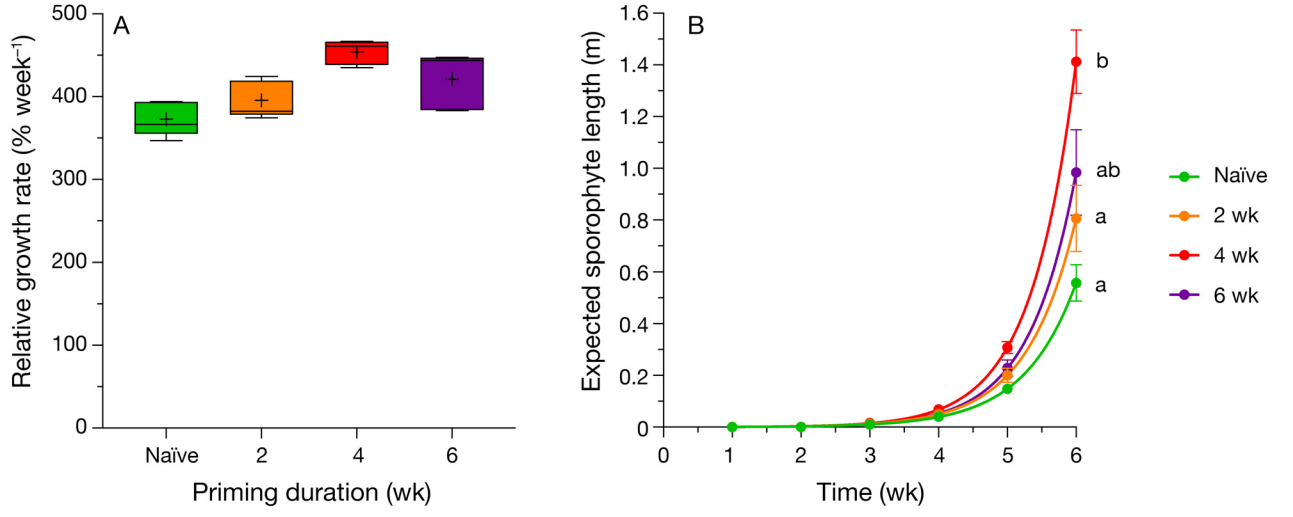

Fig. 5. (A) Relative growth rates (% week $^{-1}$) of juvenile *Saccharina latissima* sporophytes after 14 d of gametogenesis at 5°C and (B) their predicted size over time under constant growth rates from naïve gametophytes and gametophytes from 2, 4 and 6 wk of priming at 20°C (mean ± SE, n = 5). Boxplots represent the median as a line; cross as the mean number of cells per female gametophytes; interquartile range as a box; minimum and maximum values as whiskers. Letters represent significant differences between priming duration (p < 0.01, Tukey tests)

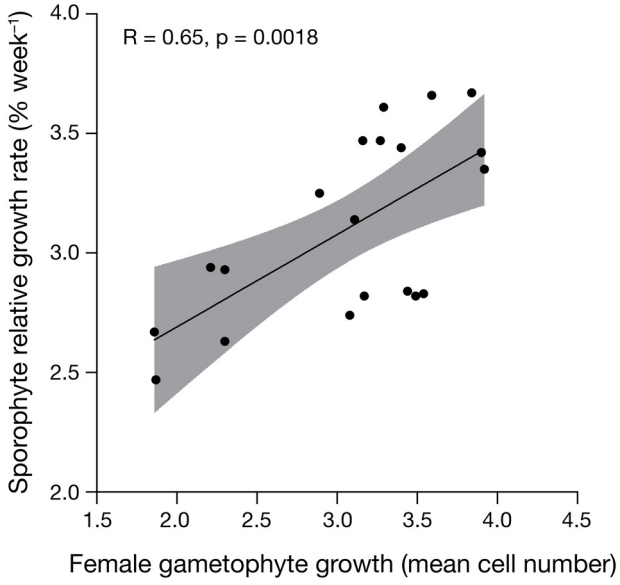

Fig. 6. Correlation plot between female gametophyte growth in the priming environment (mean cell number) and sporophyte relative growth rates (in % week^{-1}) of *Saccharina latissima* (mean, n = 5). The grey area represents the 95% confidence interval

significantly higher than those from the naïve ones at 23°C, indicating 1°C higher tolerance over 7 d (*t*-test, p = 0.046). The percentage of pigmented area of sporophytes from 4 and 6 wk priming after 7 d at 23°C was not significantly different than those from the naïve and 2 wk priming at 22°C also indicating a 1°C higher thermal tolerance over 7 d (1-way ANOVA, $F_{3,16}$ = 1.261, p = 0.321).

Table 1. Two-way repeated measures ANOVA to investigate the effects of priming duration and time on the percentage of pigmented area of *Saccharina latissima*. df_n: degrees of freedom numerator; df_d: degrees of freedom denominator. Values in **bold** are significant

Heat stress	Factor	df_n	df_d	*F*	p
20°C	Priming	3	15	2.872	0.071
	Time	4	60	3.370	**<0.05**
	Priming × time	12	60	1.302	0.241
22°C	Priming	3	16	10.187	**<0.001**
	Time	4	64	81.336	**<0.001**
	Priming × time	12	64	5.427	**<0.001**
23°C	Priming	3	16	3.633	**<0.05**
	Time	4	64	126.912	**<0.001**
	Priming × time	12	64	2.245	**<0.05**
24°C	Priming	3	15	7.524	**<0.01**
	Time	4	60	293.728	**<0.001**
	Priming × time	12	60	2.285	**<0.05**

4. DISCUSSION

This study provides a first insight into the effects of thermal priming on the most cultivated kelp species in Europe, *Saccharina latissima*. By priming parental gametophytes at 20°C for several weeks, we confirmed the hypothesis that priming can improve sporophyte formation and subsequent sporophyte growth when transferred back to cold conditions. In addition, priming of the parental gametophytes also improved the thermal tolerance of juvenile offspring sporophytes by approximately 1°C over 7 d when exposed to a lethal heat-stress gradient. Until now, it was unknown how long temperature priming must act to induce an effect. We showed that priming of gametophytes for 4 wk at 20°C was optimal for both sporophyte growth and tolerance. Kelp priming must now be tested in a more realistic farming approach to investigate whether the promising results we obtained under laboratory conditions can lead to improvements in yield for seaweed farmers (Jueterbock et al. 2021).

The novelty of our study is that we can show an increase in the sporophyte thermal tolerance of 1°C over 7 d and 4 d longer tolerance at 24°C by priming gametophytes for 4 wk at 20°C. However, as we did not post-cultivate sporophytes in favorable temperatures, we could not verify whether regrowth could take place. This is the first time the thermal tolerance has been increased using a crop enhancement technique in *S. latissima*. In terrestrial crop plants, several studies have reported an increase in tolerance at various levels (molecular, metabolic pathways, photosynthetic and physiological performances; Liu et al. 2022) following thermal priming but, to our knowledge, no studies describe an increase in survival thresholds via priming in kelps. In contrast, intra- and interspecific hybridization of kelps can increase the thermal tolerance of offspring sporophytes via heterosis (Martins et al. 2019, Wang et al. 2020, Liesner et al. 2022). Although promising, these hybridization techniques do not comply with current ethical standards and regulations for the introduction of foreign genotypes into the environment, at least in Europe (Fredriksen & Sjøtun 2015, Ramsay et al. 2022, Vissers et al. 2023). Thus, increasing species' thermal tolerance through priming could secure food production in areas that are subject to

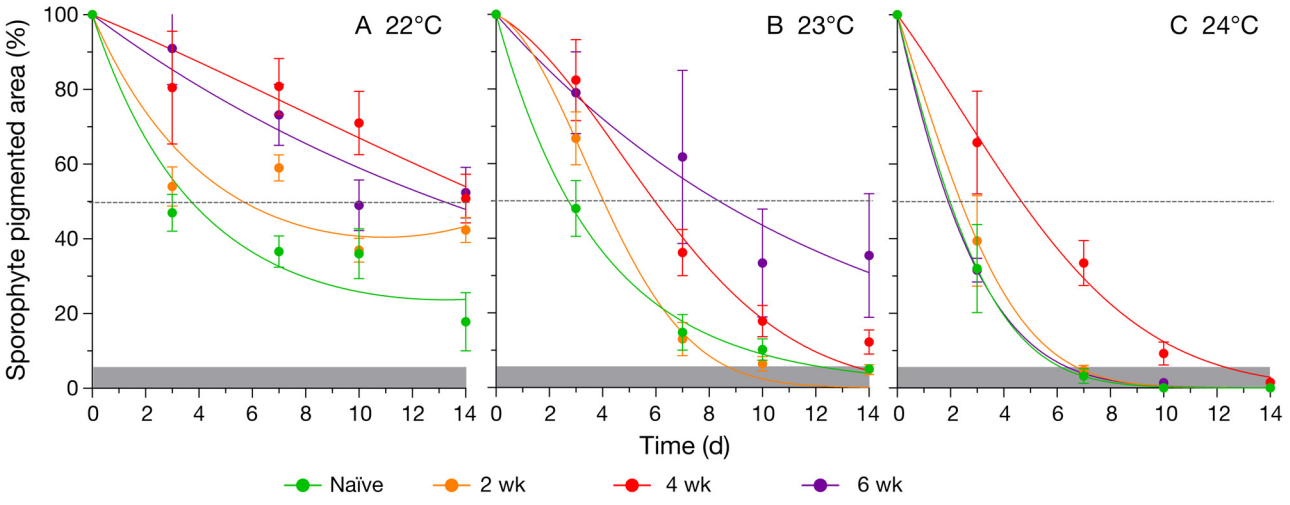

Fig. 7. *Saccharina latissima* percentage of pigmented area over time at (A) 22°, (B) 23° and (C) 24°C for each priming duration (mean ± SE, n = 5) normalized to Day 0 and fitted with a linear quadratic model for survival. The grey area represents the 5% threshold of pigmented area below which we considered regrowth unlikely

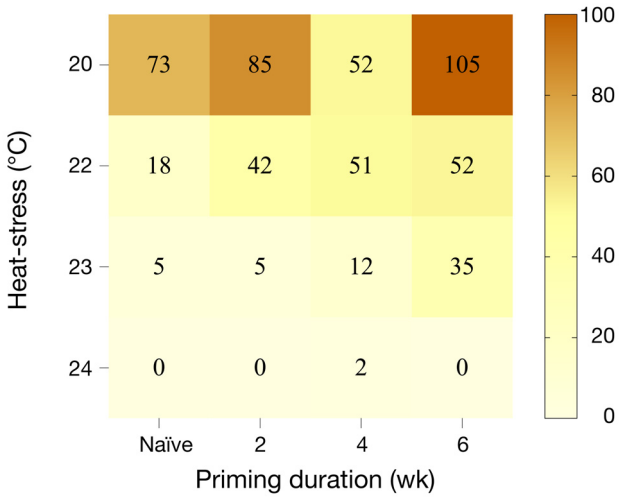

Fig. 8. Percentage of healthy pigmented area (proxy for survival) of the juvenile canopy of *Saccharina* sporophytes after 14 d in a heat-stress gradient of 20°, 22°, 23° and 24°C from naïve and primed parental gametophytes for 2, 4 and 6 wk at 20°C (mean, n = 5)

harsher conditions, or even maintain food production in areas where species are disappearing under the impact of climate change (Jueterbock et al. 2021).

The underlying mechanisms of priming remain to be investigated. As the same initial genetic pool (clonal material from 3 populations from a wide latitudinal gradient) was used in each treatment and the stress was moderate, it is unlikely that the priming effects observed could be explained by genetic adaptation. Previous evidence from seagrass and crop plants suggests the influence of epigenetics in priming, independent of the underlying genetic variation (Mercé et al. 2020, Jueterbock et al. 2021). Indeed, thermal priming in seagrass has been associated with regulation of epigenetics-related genes (Wang et al. 2017, Pazzaglia et al. 2022). In our experiment, we observed a positive correlation between female gametophyte growth in the priming environment and offspring sporophyte growth, suggesting that priming effects are sustained throughout ontogeny. Epigenetic marks have the potential to be transmitted from the gametophyte to the sporophytes in species with alternation of generations such as kelps (Vigneau & Borg 2021). Among the existing epigenetic marks, DNA methylation is the most stable (Jueterbock et al. 2021). However, the percentage of DNA methylation identified in kelps is low (2% in *S. japonica*, 1% in *S. latissima*; Fan et al. 2020, Scheschonk et al. 2022 respectively). Nevertheless, DNA methylation levels increased with temperature in the sporophytes of *S. latissima* and could play a role in increasing thermal tolerance (Scheschonk et al. 2022). Thus, more comprehensive studies that investigate several epigenome marks in relation to the transcriptome across life cycle stages are needed to understand the role of epigenetics in priming.

The fact that sporophyte growth is correlated with the proliferation rate of female gametophytes during priming at 20°C implies a positive maternal effect. Besides epigenetics, another mechanism that may explain this correlation could be the transfer of metabolites from female cells to the eggs. Indeed, in *Laminaria digitata*, Liesner et al. (2020) hypothesized that the amount of neutral lipids transferred to the

eggs might explain differences in sporophyte growth in a comparable experimental design, but conclusive evidence was not provided. This maternal effect could be independent from epigenetic effects, and therefore investigation of both sexes would provide a better understanding of cross-generational priming effects (Martins et al. 2019).

Although the mechanisms behind priming remain unclear, increased trait performance can be used to improve yield in kelp production. While 2 seeding methods are currently feasible for seaweed farmers — direct seeding of spores or pre-cultivation of gametophytes — only gametophytes can be easily primed in a hatchery. Since gametophytes can grow vegetatively for years under laboratory conditions (Bartsch 2018), they have the advantage of requiring less wild biomass, as sampling each year is not needed for the seeding of ropes. Furthermore, seeding spores is dependent on the fertile window of *S. latissima* (from December to February; Bartsch et al. 2008) although year-round spore production is possible in mesocosms if short day lengths are applied (Jun Pang & Lüning 2004). However, gametophyte reproduction can be triggered at any time in laboratory conditions by applying fertilizing conditions that are kelp-specific (Lüning 1980, Bartsch 2018). Therefore, sowing with gametophytes may enlarge the sporophyte growth period in the field (e.g. by deployment of seeded ropes from October onwards), which has been shown to improve yield at the time of harvest (Matsson et al. 2021, Thomas et al. 2022). In our experiment, 4 and 6 wk priming of gametophytes at 20°C produced the fastest growing offspring sporophytes. A faster growth rate due to priming may have a snowball effect on the produced biomass over time. Prediction of sporophyte length over 6 wk suggests that sporophytes from 4 wk of priming can become 2.5× longer than the naïve ones, assuming a constant growth rate and no limiting factors. These results highlight the need to follow growth over time during the exponential phase of an early life stage to allow further optimization. It is still unknown how long the priming effects in sporophytes last, but exponential growth in the wild usually lasts at least 2 mo in late spring (April to June; Handå et al. 2013, Stekoll et al. 2021). Therefore, priming in the context of seaweed cultivation does not need to last the life span of the species, but priming effects in the first months of life have the potential to double yield without requiring additional farming area.

Because of their small size, priming of gametophytes is a relatively low-cost crop enhancement technique that is easily feasible for seaweed farmers who work with hatcheries without much change in logistics. However, priming at 20°C for several weeks considerably reduces the amount of gametophyte biomass that farmers could seed over the years when compared with keeping gametophytes at 10°C. Therefore, future priming optimization studies should focus on reducing the duration of priming. In seagrass, priming for 9 d and up to 2 wk at temperatures 2–3°C higher than occurring in the wild improved growth rates and photosynthetic performance (Nguyen et al. 2020, Pazzaglia et al. 2022). In our experiment, we primed at the temperature limit of occurrence in the wild (20°C) and observed the best effects after 4 wk. We assume that priming at 23°C or even higher temperatures could possibly produce effects after a few days similar to those observed after 4 wk at 20°C. Another aspect that requires investigation is the potential beneficial effect of cold temperature on *S. latissima* as observed in *L. digitata*, another cold-temperate species with similar thermal characteristics (tom Dieck (Bartsch) 1992, Gauci et al. 2022). Priming of *L. digitata* gametophytes for 1.5 yr at 5°C improved both gametogenesis speed and the growth rates of sporophytes at extreme conditions (0° and 20°C) compared to priming at 15°C (Gauci et al. 2022). In our experiment, the growth rate of juvenile sporophytes was improved, but gametogenesis at 5°C was delayed following priming at 20°C for 4 and 6 wk. The delay in gametogenesis after warm treatments has recently been shown for *L. digitata*, which even exhibited population-specific responses (Martins et al. 2020). Faster reproduction will produce sporophytes that could start growing earlier and could thus be beneficial in the long run. Therefore, it would be interesting to compare the effects of cold and warm priming on both growth rates and thermal tolerance of *S. latissima* sporophytes.

While many modeling studies predict changes in kelp distribution in future climates, comprehensive studies that highlight the mitigation potential of crop enhancement techniques could help guide future conservation strategies. Kelp forest management is often limited to passive measures (that do not manipulate the kelp) such as reducing human pressures or controlling predator abundance (Eger et al. 2022). However, these passive measures are often not enough to restore kelp populations (Wernberg et al. 2019, Coleman et al. 2020). Restoration requires active measures, especially when the decline is attributed to increasing water temperatures and marine heatwaves above the physiological thresholds of kelps (Wernberg et al. 2016). Current active measures to re-establish kelp forests are transplanting (growing

juvenile or adult kelps on hard substrate) and seeding (dispersing the juvenile life stage; Eger et al. 2022), both of which are amenable to priming. While seeding has received less attention due to the high mortality of propagules (Schiel & Foster 2006), priming could increase the interest in this approach by providing more vigorous strains (Vanderklift et al. 2020).

A transplanting technique called 'green gravel' has been described as a novel tool to combat kelp forest decline (Fredriksen et al. 2020). It uses seeded spores on pebbles, cultivating them in laboratory conditions and dropping pebbles with young sporophytes from the surface (Fredriksen et al. 2020). Since it requires cultivation of young stages in the laboratory, priming can be combined easily with the green gravel method for restoration. Indeed, green gravel overcomes substrate limitations in situations where kelp has been replaced by turf algae and facilitates propagule dispersion (Fredriksen et al. 2020, Alsuwaiyan et al. 2022). The success of the green gravel method could be boosted via priming that enhances strain tolerance.

Unlike other restoration strategies that have high costs and are therefore difficult to scale up (e.g. building of artificial reefs; Eger et al. 2020, 2022), priming does not impose additional costs other than laboratory cultivation and could be performed easily in collaboration with seaweed farmers or hatcheries (Filbee-Dexter et al. 2022). In the future, it is likely that a combination of strategies, including priming, will be needed to meet the 'kelp forest challenge' expectation, a collaborative global movement to protect and restore 4 million ha of kelp forests (Eger et al. 2024).

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LITERATURE CITED

Alsuwaiyan NA, Filbee-Dexter K, Vranken S, Burkholz C, Cambridge M, Coleman MA, Wernberg T (2022) Green gravel as a vector of dispersal for kelp restoration. Front Mar Sci 9:910407

Bartsch I (2018) Derivation of clonal stock cultures and hybridization of kelps: a tool for strain preservation and breeding programs. In: Charrier B, Wichard T, Reddy CRK (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 insights and developments. Eur J Phycol 43:1–86

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

Cai J, Lovatelli A, Aguilar-Manjarrez J, Cornish L and others (2021) Seaweeds and microalgae: an overview for unlocking their potential in global aquaculture development. FAO Fisheries and Aquaculture Circular, Rome

Choi HG, Bekkby T, Norderhaug KM, Oug E, Rinde E, Fredriksen F (2018) Sukkertareskog i Nordsjøen og Skagerrak, Marint gruntvann. Norsk rødliste for naturtyper 2018. Artsdatabanken, Trondheim. https://artsdatabanken.no/RLN2018/342 (accessed 1 February 2024)

Chopin T, Tacon A (2021) Importance of seaweeds and extractive species in global aquaculture production. Rev Fish Sci Aquacult 29:139–148

Christie H, Fredriksen S, Rinde E (1998) Regrowth of kelp and colonization of epiphyte and fauna community after kelp trawling at the coast of Norway. Hydrobiologia 375: 49–58

Christie H, Jørgensen N, Norderhaug K, Waage-Nielsen E (2003) Species distribution and habitat exploitation of fauna associated with kelp (*Laminaria hyperborea*) along the Norwegian coast. J Mar Biol Assoc UK 83: 687–699

Coleman MA, Wood G, Filbee-Dexter K, Minne AJP and others (2020) Restore or redefine: future trajectories for restoration. Front Mar Sci 7:237

de Bettignies T, de Bettignies F, Bartsch I, Bekkby T and others (2021) Background document for kelp forests habitat. OSPAR Commission, London

Diehl N, Roleda M, Bartsch I, Karsten U, Bischof K (2021) Summer heatwave impacts on the European kelp *Saccharina latissima* across its latitudinal distribution gradient. Front Mar Sci 8:695821

Eger AM, Marzinelli E, Gribben P, Johnson CR and others (2020) Playing to the positives: using synergies to enhance kelp forest restoration. Front Mar Sci 7:544

Eger AM, Marzinelli EM, Christie H, Fagerli CW and others (2022) Global kelp forest restoration: past lessons, present status, and future directions. Biol Rev Camb Philos Soc 97:1449–1475

Eger AM, Aguirre JD, Altamirano M, Arafeh-Dalmau N and others (2024) The kelp forest challenge: a collaborative global movement to protect and restore 4 million hectares of kelp forests. J Appl Phycol 36:951–964

Ergon Å (2018) Breed4Kelp2Feed. NMBU. https://www.nmbu.no/en/research/projects/breed4kelp2feed (accessed 1 February 2024)

European Commission, Joint Research Centre (2019) Brief on algae biomass production. https://op.europa.eu/en/publication-detail/-/publication/037825ae-22d7-11ea-af81-01aa75ed71a1/language-en (accessed 8 February 2024)

Fan X, Han W, Teng L, Jiang P and others (2020) Single-base methylome profiling of the giant kelp *Saccharina japonica* reveals significant differences in DNA methylation to microalgae and plants. New Phytol 225:234–249

Filbee-Dexter K, Wernberg T, Barreiro R, Coleman M and others (2022) Leveraging the blue economy to transform marine forest restoration. J Phycol 58:198–207

Forbord S, Steinhovden KB, Solvang T, Handå A, Skjermo J (2020) Effect of seeding methods and hatchery periods on sea cultivation of *Saccharina latissima* (Phaeophyceae): a Norwegian case study. J Appl Phycol 32:2201–2212

Fortes MD, Lüning K (1980) Growth rates of North Sea

macroalgae in relation to temperature, irradiance and photoperiod. Helgol Meeresunters 34:15–29
Franke K, Liesner D, Heesch S, Bartsch I (2021) Looks can be deceiving: contrasting temperature characteristics of two morphologically similar kelp species co-occurring in the Arctic. Bot Mar 64:163–175
Fredriksen S, Sjøtun IH (2015) Risikovurdering ved utsetting av ikke–stedegen tare. Rapport M-299. Miljødirektoratet, Universitetet i Oslo og Universitetet i Bergen
Fredriksen S, Filbee-Dexter K, Norderhaug KM, Steen H and others (2020) Green gravel: a novel restoration tool to combat kelp forest decline. Sci Rep 10:3983
Gauci C, Bartsch I, Martins N, Liesner D (2022) Cold thermal priming of *Laminaria digitata* (Laminariales, Phaeophyceae) gametophytes enhances gametogenesis and thermal performance of sporophytes. Front Mar Sci 9:862923
Handå A, Forbord S, Wang X, Broch OJ and others (2013) Seasonal- and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in Norway. Aquaculture 414-415:191–201
Hobday AJ, Alexander LV, Perkins SE, Smale DA and others (2016) A hierarchical approach to defining marine heatwaves. Prog Oceanogr 141:227–238
Hu ZM, Shan TF, Zhang J, Zhang QS and others (2021) Kelp aquaculture in China: a retrospective and future prospects. Rev Aquacult 13:1324–1351
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
Jun Pang S, Lüning K (2004) Breaking seasonal limitation: year-round sporogenesis in the brown alga *Laminaria saccharina* by blocking the transport of putative sporulation inhibitors. Aquaculture 240:531–541
Lewis RJ, Green MK, Afzal ME (2013) Effects of chelated iron on oogenesis and vegetative growth of kelp gametophytes (Phaeophyceae): effects of iron on kelp gametophytes. Phycol Res 61:46–51
Li Y, Umanzor S, Ng C, Huang M and others (2022) Skinny kelp (*Saccharina angustissima*) provides valuable genetics for the biomass improvement of farmed sugar kelp (*Saccharina latissima*). J Appl Phycol 34:2551–2563
Liesner D, Shama LNS, Diehl N, Valentin K, Bartsch I (2020) Thermal plasticity of the kelp *Laminaria digitata* (Phaeophyceae) across life cycle stages reveals the importance of cold seasons for marine forests. Front Mar Sci 7:456
Liesner D, Pearson GA, Bartsch I, Rana S and others (2022) Increased heat resilience of intraspecific outbred compared to inbred lineages in the kelp *Laminaria digitata*: physiology and transcriptomics. Front Mar Sci 9:862923
Lindell S (2021) Woods Hole Oceanographic Institution — kelp breeding. http://arpa-e.energy.gov/mariner-annual-review-2021/woods-hole-oceanographic-institution-kelp-breeding (accessed 1 February 2024)
Liu H, Able AJ, Able JA (2022) Priming crops for the future: rewiring stress memory. Trends Plant Sci 27:699–716
Lüning K (1980) Critical levels of light and temperature regulating the gametogenesis of three *Laminaria* species (Phaeophyceae). J Phycol 16:1–15
Lüning K (1984) Temperature tolerance and biogeography of seaweeds: the marine algal flora of Helgoland (North Sea) as an example. Helgol Meeresunters 38:305–317
Lüning K, Dring MJ (1975) Reproduction, growth and photosynthesis of gametophytes of *Laminaria saccharina* grown in blue and red light. Mar Biol 29:195–200
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
Martins N, Pearson GA, Gouveia L, Tavares AI, Serrão EA, Bartsch I (2019) Hybrid vigour for thermal tolerance in hybrids between the allopatric kelps *Laminaria digitata* and *Laminaria pallida* (Laminariales, Phaeophyceae) with contrasting thermal affinities. Eur J Phycol 54:548–561
Martins N, Pearson GA, Bernard J, Serrão EA, Bartsch I (2020) Thermal traits for reproduction and recruitment differ between Arctic and Atlantic kelp *Laminaria digitata*. PLOS ONE 15:e0235388
Matsson S, Metaxas A, Forbord S, Kristiansen S, Handå A, Bluhm BA (2021) Effects of outplanting time on growth, shedding and quality of *Saccharina latissima* (Phaeophyceae) in its northern distribution range. J Appl Phycol 33: 2415–2431
Mercé C, Bayer PE, Tay Fernandez C, Batley J, Edwards D (2020) Induced methylation in plants as a crop improvement tool: progress and perspectives. Agronomy (Basel) 10:1484
Neiva J, Paulino C, Nielsen MM, Krause-Jensen D and others (2018) Glacial vicariance drives phylogeographic diversification in the amphi-boreal kelp *Saccharina latissima*. Sci Rep 8:1112
Nguyen HM, Kim M, Ralph PJ, Marín-Guirao L, Pernice M, Procaccini G (2020) Stress memory in seagrasses: first insight into the effects of thermal priming and the role of epigenetic modifications. Front Plant Sci 11:494
Oliver ECJ, Donat MG, Burrows MT, Moore PJ and others (2018) Longer and more frequent marine heatwaves over the past century. Nat Commun 9:1324
Pazzaglia J, Badalamenti F, Bernardeau-Esteller J, Ruiz JM, Giacalone VM, Procaccini G, Marín-Guirao L (2022) Thermo-priming increases heat-stress tolerance in seedlings of the Mediterranean seagrass *Posidonia oceanica*. Mar Pollut Bull 174:113164
Provasoli L (1968) Media and prospects for the cultivation of marine algae. In: Hattori A, Watanabe A (eds) Cultures and collections of algae. Proc US–Japan Conf, 12–15 September 1966, Hakone. Japan Society of Plant Physiology, Kyoto, p 63–75
R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
Ramsay T, Robinson B, Coche I, Hackett K, Emerson C (2022) Ethical aspects of GMO regulation in the EU: regulating new plant breeding techniques as GM has negative effects on sustainability, diversity and inclusion. EMBO Rep 23:e55583
Scheschonk L, Bischof K, Kopp M, Jueterbock A (2022) Differences by origin in methylome suggest eco-phenotypes in the kelp *Saccharina latissima*. Evol Appl 16:262–278
Schiel DR, Foster MS (2006) The population biology of large brown seaweeds: ecological consequences of multiphase life histories in dynamic coastal environments. Annu Rev Ecol Evol Syst 37:343–372
Schindelin J, Arganda-Carreras I, Frise E, Kaynig V and others (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682
Shan T, Li Q, Wang X, Su L, Pang S (2019) Assessment of the

genetic connectivity between farmed populations on a typical kelp farm and adjacent spontaneous populations of *Saccharina japonica* (Phaeophyceae, Laminariales) in China. Front Mar Sci 6:494

Stekoll MS, Peeples TN, Raymond AET (2021) Mariculture research of *Macrocystis pyrifera* and *Saccharina latissima* in Southeast Alaska. J World Aquacult Soc 52: 1031–1046

Stévant P, Rebours C, Chapman A (2017) Seaweed aquaculture in Norway: recent industrial developments and future perspectives. Aquacult Int 25:1373–1390

Thomas JB, Sterner M, Nylund GM, Albers E and others (2022) The effects of cultivation deployment- and harvest-timing, location and depth on growth and composition of *Saccharina latissima* at the Swedish west coast. Aquaculture 559:738443

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

Umanzor S, Li Y, Bailey D, Augyte S and others (2021) Comparative analysis of morphometric traits of farmed sugar kelp and skinny kelp, *Saccharina* spp., strains from the Northwest Atlantic. J World Aquac Soc 52:1059–1068

Vanderklift MA, Doropoulos C, Gorman D, Leal I and others (2020) Using propagules to restore coastal marine ecosystems. Front Mar Sci 7:862923

Vea J, Ask E (2011) Creating a sustainable commercial harvest of *Laminaria hyperborea*, in Norway. J Appl Phycol 23:489–494

Vigneau J, Borg M (2021) The epigenetic origin of life history transitions in plants and algae. Plant Reprod 34: 267–285

Vissers C, Lindell S, Nuzhdin S, Almada A, Timmermans K (2023) Using sporeless sporophytes as a next step towards upscaling offshore kelp cultivation. J Appl Phycol 36:313–320

Wang X, Liu F, Jiang D (2017) Priming: a promising strategy for crop production in response to future climate. J Integr Agric 16:2709–2716

Wang X, Yao J, Zhang J, Duan D (2020) Status of genetic studies and breeding of *Saccharina japonica* in China. J Oceanol Limnol 38:1064–1079

Wernberg T, Bennett S, Babcock RC, de Bettignies T and others (2016) Climate-driven regime shift of a temperate marine ecosystem. Science 353:169–172

Wernberg T, Krumhansl K, Filbee-Dexter K, Pedersen MF (2019) Status and trends for the world's kelp forests. In: Sheppard C (ed) World seas: an environmental evaluation. Academic Press, London, p 57–78

Appendix.

Table A1. AWI culture number and origins

Population	Sex	Isolation year	AWI culture number
Oslo	♂	1974	3300
	♀		3301
Bergen	♂	1975	3305
	♀		3306
Spitsbergen	♂	2011	3491
	♀		3492
	♂	2011	3493
	♀		3494
	♂	2015	3497
	♀		3498
	♂	2015	3499
	♀		3500

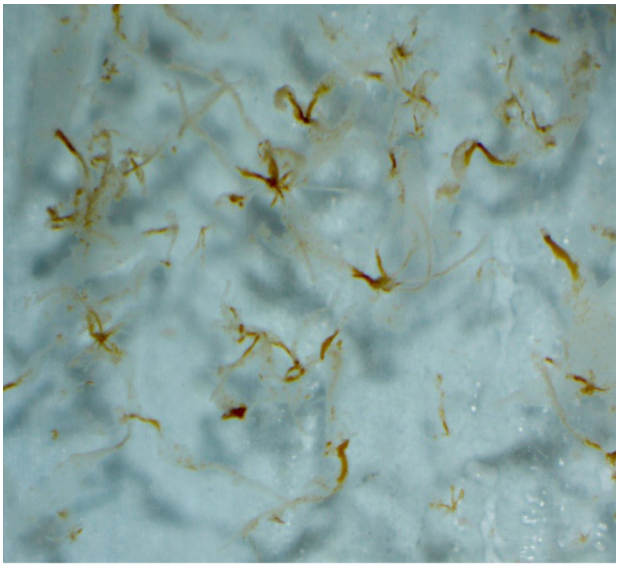

Fig. A1. Naïve sporophytes after 14 d at 23°C with healthy meristem but a mainly bleached blade (pigmented area: 6.6%)

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