Journal of Applied Phycology
https://doi.org/10.1007/s10811-025-03454-8

RESEARCH

Predicting heat tolerance in sugar kelp juvenile sporophytes via gametophyte heat stress testing

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Received: 12 May 2024 / Revised: 17 January 2025 / Accepted: 17 January 2025

Abstract

Warming sea temperatures are threatening global kelp populations, including sugar kelp (*Saccharina latissima*), one of the most commonly farmed kelp species. For the future of kelp aquaculture and restoration, we must identify individuals with natural adaptations to cope with heat. If heat tolerance of adult kelp can be predicted by assessment at the early-life gametophyte stage (prior to fertilization), then we can accelerate breeding of heat-tolerant strains. We developed a high-throughput method for assessing gametophyte physiological stress under heat and conducted an experiment to generate predictions of heat-tolerant strains. Gametophyte heat tolerance was assessed for 93 distinct genotypes by exposing them to temperatures representing current (12°C) and future high (24°C) temperatures in the Gulf of Maine, U.S.A., and measuring their photosynthetic performance (as chlorophyll *a* fluorescence) using a plate-reading spectrofluorometer. Individual tolerance to heat, which was determined by comparing post-heat to baseline fluorescence values, ranged from 4% to 100%. We then conducted a heat stress experiment with juvenile sporophytes made by crossing male and female suspected heat-tolerant gametophytes, and male and female suspected intolerant gametophytes. Juvenile sporophytes from 7 unique crosses were exposed to control (12°C) and warming (up to 22°C) temperatures and their growth and percent degradation were monitored. Our results indicate that we can identify heat-tolerant gametophytes that, when crossed, yield juvenile sporophytes that have greater growth under heat stress than sporophyte progeny of predicted intolerant gametophytes. These results indicate the potential to create heat-tolerant sporophytes by knowing only the gametophyte performance under heat stress.

Keywords Aquaculture · Climate change · Gametophyte · Heat tolerance · Kelp · Phaeophyceae · Selective breeding

Introduction

Algae, primarily seaweed, production has grown more than seven-fold globally since 1990 (FAO 2022), with increased demand for uses as food, food additives (gels/thickeners), pharmaceuticals, cosmetics, and fertilizers. Seaweed aquaculture has gained popularity not only for products derived from seaweed but also for its potential to mitigate effects of climate change, as it has a lower carbon footprint than terrestrial crop agriculture and can be used to make products including biofuel and methane-lowering cattle feed (reviewed in Duarte et al. 2017). Farming seaweed is important for the future of the resource, as many wild populations are facing overexploitation and relying on wild harvest is unsustainable for current and future demand for the resource as well as environmental health (Mazarrasa et al. 2014; Buschmann et al. 2017). Dozens of species of seaweed are cultivated for different uses based on their distinctive tastes, nutrient profiles, and chemical properties. Of the cultivated seaweed species, kelps (Laminariales) have the capacity to produce the most biomass per farmed area annually. At large scales (e.g. around 73,000 km^2) with purposeful conveyance to the deep ocean, kelp could sequester one-tenth of a gigatonne of CO_2 per year (NASEM 2022). Additionally, since kelp have a biphasic life cycle with a free-living gamete-producing life stage (gametophyte), genetic combinations can be easily manipulated by crossing specific male and female gametophytes, allowing for selective breeding for traits of interest.

While kelp farming may help reduce global carbon emissions, the effects of climate change are also impacting

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Published online: 07 February 2025

the kelp itself. Wild kelp is the seed source for genetic diversity for farmed kelp, but distributional shifts due to warming are likely to occur (Smale 2020; Taylor-Robinson et al. 2024). Casado-Amezúa et al. 2019 estimated the range contraction of *Saccharina latissima* in the southern part of the NE Atlantic from the 1980's-90's to 2013–2016 was 6%, and up to 45% for other cold-temperate brown algae. The Gulf of Maine region is changing faster than most other places in the world, and sea surface temperatures could warm 0.5–3.5°C by 2100 (Bricknell et al. 2021). As a result of this warming, farmed kelp will experience a shortened growing season, and thus reduced yield, since it will have to be planted later in the fall and harvested sooner in the spring when water temperatures are cool enough to support healthy growth. Warming also restricts the range of farmable areas for some kelp populations, as evidenced by declines in natural kelp forests experiencing increased temperatures in both the Northern and Southern Hemispheres (Filbee-Dexter et al. 2020; Wernberg et al. 2016; Cavanaugh et al. 2019; Berry et al. 2021). Growth and biofouling of farmed kelp can be affected by temperature, along with other environmental factors such as salinity, depth, and light (Forbord et al. 2020). As the seaweed aquaculture industry continues to grow each year worldwide, we need to consider how to build resilience in the industry in the face of climate change.

Heat tolerance in terrestrial crops has been defined as acclimation leading to reduced tissue damage in high temperatures (Hemantaranjan et al. 2018) or producing an "economically significant yield" under heat stress (Bita and Gerats 2013) which can be due to increased photosynthetic rate, transpiration rate, or cell membrane thermostability (Marcum 1998; Liu and Huang 2008; Rajametov et al. 2021). Previous work to identify heat tolerance in crops and kelp have included physiological monitoring of adult tissue. Evidence of damaging temperatures in crop plants is usually observed as scorching of leaves, tissue senescence, and inhibition of root growth (Bita and Gerats 2013) and can lead to significant loss in yield (Prasad et al. 2017). For kelp sporophytes, methods for identifying thermal tolerance include measuring survival, growth, photosynthetic performance, or xanthophyll pigment response of individuals under heat stress (Gao et al. 2016; Nepper-Davidsen et al. 2019; Liesner et al. 2020; Niedzwiedz et al. 2022; Harris et al. 2023; Gauci et al. 2024), or measuring the density of microscopic sporophytes or the relative growth rate of macroscopic sporophytes after heat exposure (Martins et al. 2019; Schimpf et al. 2022). The physiological responses at the gametophyte stage of kelp are harder to measure, especially as their irregular shape complicates measuring growth, but previous studies have used gametophyte size and density to determine optimum growing temperature (Mohring et al. 2014), or fluorometry to estimate photosynthetic efficiency in kelp gametophytes when exposed to heat stress (Becheler et al. 2022; Strasser et al. 2022; Harden et al. 2024).

Breeding for heat tolerance may depend on genetic approaches to map genes that confer thermotolerance (Asthir 2015). If specific genes can be identified at the DNA or RNA level, this opens possibilities for selective breeding for aquaculture. Some responses to heat stress are at the RNA level, such as increased expression of heat-shock proteins as an adaptive strategy to cope with thermal stress (Henkel and Hofmann 2008; Asthir 2015). For example, evidence for variation in heat shock protein expression by geographic region has been observed in the kelp *Laminaria digitata* (King et al. 2019). However, many mechanisms to cope with heat stress are regulated at the DNA level (Wahid et al. 2007), and with sufficient genetic diversity in a population it is likely that some individuals already have adaptations to cope with heat. Previous studies have observed high variation in thermal stress response across genotypes in kelps including *Ecklonia radiata* (Mabin et al. 2019; Alsuwaiyan et al. 2021), *L. digitata* (King et al. 2019; Schimpf et al. 2022), and *Macrocystis pyrifera* (Becheler et al. 2022). Identifying individuals with heat-tolerance adaptations will provide a pathway for breeding heat-tolerant kelp.

Much of the previous research on heat tolerance in kelp has focused on the adult sporophyte, but breeding depends on manipulation at the gametophyte stage, and the relationship between gametophyte and sporophyte heat tolerance has not yet been established. The free-living early life stage of kelps offers an opportunity to test for heat tolerance phenotypes at the gametophyte stage and potentially predict sporophyte heat tolerance from gametophytes. Studies measuring thermal tolerance in gametophytes in relation to sporophytes are often in the context of priming, that is, examining the effect of exposing the gametophyte to extreme cold (Gauci et al. 2022) or warm (Quigley 2018; Schimpf et al. 2022; Gauci et al. 2024) temperatures on the performance of sporophyte offspring under those same temperatures. Gametophyte heat tolerance is typically higher than that of sporophytes (Bolton and Luning 1982; Schiel and Foster 2015; Franke et al. 2021), which can be important for the persistence of kelp beds through summer temperatures. A recent study on *M. pyrifera* found genetic similarities between gametophytes that exhibited heat tolerance--measured as chlorophyll fluorescence when cultivated under heat stress--and sporophytes that grew well in warm ocean waters, suggesting a genetic basis for heat tolerance (Harden et al. 2024). However, it is unclear if or how heat tolerance is inherited, and whether heat tolerance at the gametophyte stage can be predictive of heat tolerance at the sporophyte stage.

Our study aimed to address whether we can identify heat tolerance in sugar kelp (*Saccharina latissima*) and skinny kelp (*Saccharina angustissima*) at the gametophyte stage that relates to heat tolerance in their sporophyte progeny.

While *S. latissima* and *S. angustissima* have been previously distinguished taxonomically based on blade morphology, more recent work showed that the two putative species do not any exhibit clear genetic separation, and we treated them as conspecific for the purposes of our breeding program. Using genome-wide high-density markers, Mao et al. (2020) found that there was greater genetic differentiation among populations of *S. latissima* from the Gulf of Maine and southern New England than between *S. latissima* and *S. angustissima*. In addition, *S. latissima* and *S. angustissima* are interfertile (Augyte et al. 2018), and do not exhibit significant differences in yield when cultivated on ocean farms as either pure-crosses or hybrids (Umanzor et al. 2021; Li et al. 2022). Therefore, we included *S. angustissima* gametophytes in our heat stress testing along with *S. latissima* gametophytes. We conducted two experiments to address the following questions: 1) What is the variation in response to heat stress among gametophytes sourced from wild populations in the Gulf of Maine and southern New England? 2) How do juvenile sporophytes from crosses of suspected heat-tolerant gametophytes and suspected intolerant gametophytes compare in growth and tissue health when cultivated in heat and in control (cold) conditions? To answer these questions, we first developed a high-throughput method for screening gametophytes for heat tolerance by measuring their change in fluorescence over time when exposed to heat stress. Next, we used these results to identify high-performing and low-performing gametophytes under heat stress, made crosses within each of these groups, and measured the growth response of the resulting juvenile sporophytes under control and heat conditions.

Materials & methods

Origin of gametophytes

Woods Hole Oceanographic Institution has a repository of gametophyte cultures from 29 populations ranging from New York to Maine, which allows our research group to make individual crosses of males and females to yield specific genotypes (see Mao et al. 2020; Umanzor et al. 2021; Huang et al. 2023). The gametophytes used for this study were isolated from wild sporophytes sourced from populations in nearshore waters between New York and Maine, U.S.A. in 2018, and were kept in vegetative culture under low intensity red light (10 to 30 µmol photons m^{-2} s^{-1}, 12:12 L:D photoperiod) and ~12°C for approximately 4.5 years prior to use in this study. In the first experiment testing heat tolerance of gametophytes, we used gametophytes from 14 populations (Table 1). We selected gametophytes from these populations to represent the genetic diversity of Northeastern U.S. wild sugar kelp populations (see map in Mao et al. 2020). Winter temperatures are not meaningfully different between southern New England and Gulf of Maine, but summer sea surface temperatures are significantly different with highs around 23°C in Connecticut and New York (Gruenburg et al. 2021), and 18°C in Maine (Gulf of Maine Research Institute 2023). These temperatures are respectively 1°C and 2°C higher than 20 years ago.

In the second experiment testing heat tolerance of juvenile sporophytes, we used gametophytes from 6 populations (Table 1), which were selected based on their heat tolerance performance (see below). Details about the number of sporophytes sampled per population and number of male and

Table 1 Sites of origin for *Saccharina latissima* and *S. angustissima* gametophytes used in heat stress experiments. All populations listed were used in the gametophyte heat stress experiment; populations indicated with * in the final column were used to make crosses for the juvenile sporophyte heat stress experiment

Population	Site code	Latitude	Longitude	* = used for juvenile sporophyte heat stress experiment
Thimble Island	TI	41.25728	−72.76588	
Black Ledge	BL	41.30745	−72.07054	
Pine Island	PI	41.312122	−72.06127	
Fort Wetherill	FW	41.477127	−71.36107	
Cape Cod Canal	CC	41.77382	−70.49945	*
New Castle	NC	43.05767	−70.72742	*
Fort Stark	JS	43.059444	−70.71174	*
Nubble Lighthouse	NL	43.16692	−70.59145	
University of New Hampshire Dock	OD	43.07221	−70.71047	
Sulivans Falls	SF	44.51997	−68.220695	*
Casco Bay	CB	43.723248	−69.994223	*
Orr's Island Bridge	OI	43.79541	−69.94545	*
Lubec Lighthouse	LL	44.841152	−66.97825	
Lubec Dock	LD	44.86168	−66.9821	

female gametophytes collected per sporophyte can be found in Supplementary Material, Tables S1 and S2.

Generating predictions of gametophyte heat tolerance through heat stress testing

We selected 93 gametophytes (93 distinct genotypes) from our germplasm collection to test for heat tolerance. For each gametophyte, we plated ~1.25 mg (wet weight) of clonal fragments per each of three wells in a 96 well plate (in predetermined random locations), with 250 µL of seawater + f/2 culture medium per well (Proline f/2 algae food, which contains vitamin and mineral content ratios equal to the formulation described by Guillard (1975)). Each gametophyte had 3 replicate wells for each of 2 treatments (558 samples total), a control (12°C, ~30 µmol photons m^{-2} s^{-1} red LED light, 12:12 L:D photoperiod) and a heat treatment (24°C, ~30 µmol photons m^{-2} s^{-1} red LED light, 12:12 L:D photoperiod). Light measurements were measured with a LI-1400 Light Sensor Logger (LI-COR Environmental, USA), and temperatures were recorded with HOBO temperature loggers (Onset Computer, USA). The gametophytes in the 24°C treatment were subjected to gradually increasing temperatures (1–2°C per day over 11 days) up to 24°C on day 12, then maintained at 24°C for two weeks, and then put into 12°C for three weeks to recover prior to testing fertility. Every two to four days throughout the heating portion of the experiment, we measured the fluorescence intensity of the gametophytes using a SpectraMax M3 reader (Molecular Devices, USA) with excitation at 440 nm and emission at 684 nm, representing the wavelengths at which chlorophyll *a* absorbs light and emits fluorescence for Photosystem II (Eullaffroy and Vernet 2003; Lee et al. 2020). We also included three ethanol-treated gametophyte replicates per treatment and six filtered seawater replicates per treatment to obtain signals for a dead gametophyte and background fluorescence. We measured the reproductive success of the gametophytes after three weeks of recovery post-heat stress by removing 100 µL of seawater from each well and introducing 100 µL of a mix of opposite-sex gametophytes (three males for each female tested or five females for each male tested) into each well. A gametophyte was considered fertile if at least one normal (non-parthenogenic) sporophyte was produced in the well. The male and female gametophytes used for fertility testing were selected based on prior evidence of high fertility (see Supplementary Information, Table S3 for list of gametophytes).

To quantify performance under heat stress, we calculated the percent change in fluorescence for each well from the start of the experiment (Day 1; January 12, 2023) to the end of the heat-stress period (Day 26; February 6, 2023), and then obtained the mean percent change in fluorescence for each gametophyte genotype. When selecting our highest and lowest performing gametophytes, we eliminated any gametophyte that declined more than 50% (average of three replicates) in the control treatment, had an initial fluorescence intensity equal to or lower than the lowest mean value for the ethanol-treated gametophytes, or failed to produce a sporophyte in the fertility test in the control treatment. For the remaining gametophytes, the "percent tolerance" for each gametophyte was defined as the inverse of the mean percent decline in fluorescence. Our most heat-tolerant gametophytes were classified as those with the highest percent tolerance, and our least heat-tolerant gametophytes were those with the lowest percent tolerance.

Testing predictions with juvenile sporophytes

We selected 6 high-performing gametophytes (3 male, 3 female) to create 3 unique crosses (Tolerant prediction group), and 8 low-performing gametophytes (4 male, 4 female) to create 4 unique crosses (Intolerant prediction group) based on the results of the gametophyte heat stress testing (Table 2). We followed the procedure for creating crosses and cultivating juvenile sporophytes described by Umanzor et al. (2021). To create each cross, we fragmented the gametophytes in a 1.5 mL microcentrifuge tube with a pestle and mixed 5 mg of the male biomass and 10 mg of the female biomass in a square Petri dish (100 cm^2) with 60 mL of filtered seawater + f/2 culture

Table 2 Crosses made with selected gametophytes from heat stress experiment for juvenile sporophyte experiment. Gametophytes codes are written as SL18 (*Saccharina latissima,* 2018) or SA18 (*Saccharina angustissima,* 2018) followed by the two-letter site code (see Table 1), the wild sporophyte identification number, and the gametophyte identification number with FG=female gametophyte and MG=male gametophyte

Cross	Prediction	Female eametophvte	Male eametophvte	Female % tolerant	Male % tolerant
144	Tolerant	SLI 8-CC-5-FG3	SL18-OI-16-MG1	72.4	47.7
202	Tolerant	SLI 8-JS-9-FG3	SLI 8-JS-19-MG1	128.5	100.3
213	Tolerant	SL18-NC-10-FG1	SLI8-JS-6-MG1	100.2	74.9
161	Intolerant	SL18-CC-6-FG3	SL18-CC-3-MG1	22	4.2
192	Intolerant	SL18-JS-16-FG1	SLI8-JS-6-MG2	26.7	13.7
196	Intolerant	SLI 8-JS-5-FG1	SA18-CB-10-MG1	25.5	14.9
296	Intolerant	SL18-SF-13-FG2	SL18-Ol-15-MG1	21.9	16.5

medium. To induce fertilization, we cultured the gametophytes in a 12°C growth chamber with irradiance of 60 µmol photons m^{-2} s^{-1} full-spectrum LED light (gradually increased from 20 µmol photons m^{-2} s^{-1} over the first week) for approximately four weeks, with a 12:12 L:D photoperiod for the first week and then a 16:8 L:D photoperiod for the remaining weeks.

Once fertilization was complete and embryonic sporophytes were visible under the microscope (approximately 4–5 weeks after inducing fertilization, depending on individual cross development), we used a 20 µm filter to concentrate the juvenile sporophytes and a calligraphy brush to paint the material onto a 1-m section of 2-mm diameter Kuralon twine wrapped around a microscope slide, and placed the slide into a clear 300 mL polycarbonate box (bioWORLD, USA) filled with seawater + f/2 culture medium. The slides were cultured in the growth chamber (12°C, 12:12 L:D) for 5 weeks, until the sporophytes were approximately 1 cm in length. We exchanged half of the water in each polycarbonate box with new seawater + f/2 culture medium once per week. Light intensity was either 150 or 50 µmol photons m^{-2} s^{-1} full-spectrum LED light initially as we maintained replicate microscope slides of each cross at both high and low light intensity to ensure that, in the case of differing growth rates, all crosses would reach 1 cm long sporophytes at approximately the same time. For the final two weeks of cultivation prior to starting the heat stress experiment, all sporophytes were maintained at 50 µmol photons m^{-2} s^{-1}. From each of the 3 predicted tolerant and 4 predicted intolerant crosses, we selected 12 healthy sporophytes (~1 cm in length) from each cross and placed each one into a test tube with culture media. For each cross, six of the sporophytes were assigned to the control treatment and six were assigned to the heat treatment (see below).

We conducted the experiment in two incubators. The control incubator (Control) was maintained at constant 12°C, and the temperature in the heat treatment incubator (Heat) was gradually increased from 12°C to 22°C over 3 weeks. We increased the temperature gradually to avoid shocking the blades through an unnatural temperature change, and to align our methods with those of previous studies on kelp sporophyte heat tolerance (e.g. Nepper-Davidsen et al. 2019; Diehl et al. 2021; Niedzweidz et al. 2022). The average irradiance was 125 µmol photons m^{-2} s^{-1} white fluorescent light in the Control and 133 µmol photons m^{-2} s^{-1} white fluorescent light in the Heat treatment.

At each measurement date, we took digital photos of each blade over a white LED backlit platform to standardize light across photos and enhance ability to see degradation, and we included a transparent ruler in each photo. We took photos at 3 timepoints: after 2 days of acclimation in incubators set to 12°C ("T0"), after 2–3 days at 20°C ("T1"), and after 4–5 days at 22°C ("T2"). For logistical reasons, replicates 1–3 of each cross were photographed on the first day of each timepoint and replicates 4–6 were photographed on the second day of each timepoint, except for T0 where all replicates 1–6 were photographed on the same day. One blade (Cross 192, Control replicate #3) was damaged during handling after photographing at T1, so it could not be measured at T2.

We analyzed the photos using the digital image software, Fiji (ImageJ), to calculate the area. First, we calibrated the scale of the image based on the length of 1 mm on the ruler included in the photo. We then used the Adjust Color Threshold function to isolate the blade from the background. We used the Paintbrush tool to remove the stipe and holdfast to standardize measurements of the blade only across images. From this selected blade image, we measured the total area in mm^2. We calculated the percent change in total area for each blade from T0 to T1 and from T0 to T2 as $((T_{final}$ area - $T_{initial}$ area)/$T_{initial}$ area)×100.

We quantified the percentage of healthy tissue in each blade by conducting a color analysis using Matlab and the selected blade images created in Fiji. We used the transmittance of blue light through the blade tissue to quantify the percentage of degraded tissue on each blade. First, we standardized the overall luminance across the entire microscope slide by the brightest cluster of luminance values (the backlight) in each image using the *imsegkmeans* function (number of total clusters equals five). Then we calculated the transmittance of the red, green, and blue color bands for each pixel in the blade area. Since chlorophyll *a* absorbs light primarily in blue wavelengths, more degraded tissue absorbs less blue light and consequently has higher blue light intensity values (see Supplementary Material, Figure S1). For each blade, we calculated the proportion of pixels within the blade area with blue light transmittance values that were >50% and categorized this value as the proportion of degraded tissue. We calculated the proportion of healthy tissue as (1 - proportion of degraded tissue), and we calculated the area of healthy tissue for each blade as (total area in mm^2 * proportion of healthy tissue); see Fig. 1 for examples. Finally, we calculated the percent change in healthy area for each blade from T0 to T1 and from T0 to T2 as described for total area, above. Over the course of the experiment, a single sample--a replicate of Cross 192 in the Control--was damaged after taking measurements at T1 and therefore was not measured at T2.

Statistical analyses

In the gametophyte heat stress experiment, we assessed the effect of heat on gametophyte survival by conducting a paired t-test on the percent change in fluorescence from the start of the experiment to the end of the heat stress period for each of the 93 gametophytes between the 12°C control and the 24°C treatment.

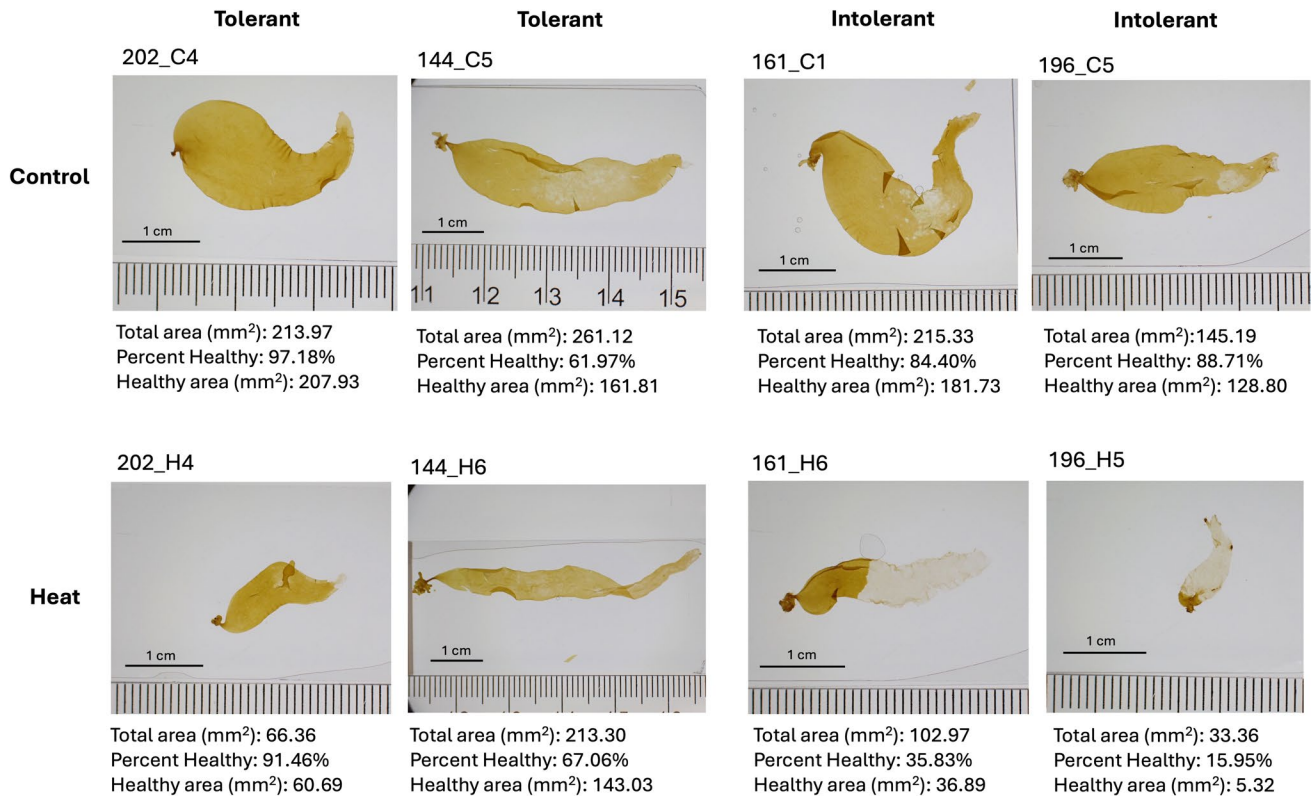

Fig. 1 Selected photos of *Saccharina latissima* juvenile sporophytes in the Control (**top row**) and Heat (**bottom row**) experimental treatments, exhibiting different amounts of degraded tissue on blades at timepoint T2, when the Heat treatment was at 22°C. Photos are labeled in the format [Cross#]_[Treatment][Replicate]. The images in the first two columns are samples from the predicted Tolerant group, and images in the last two columns are from the predicted Intolerant group

In the juvenile sporophyte heat stress experiment, we compared blade growth (total area) and growth of healthy tissue (healthy area) between prediction groups and treatments using mixed models via the "lme4" package in R with prediction, treatment, and time as fixed effects and cross nested within prediction group as a random effect for the response variables of percent change in total area and percent change in healthy area (both square-root transformed). Post-hoc pairwise comparisons were conducted using estimated marginal means (EMMs) with contrasts via the "`emmeans`" package in R. We also used a t-test to compare the mean proportion of total growth during the experiment that was achieved between T0 and T1 among all crosses in the Heat versus Control.

Results

Gametophyte heat stress testing and selection for prediction groups

The mean decline in fluorescence of all 93 gametophytes from the baseline to the end of the experimental period was significantly greater in the 24°C treatment compared to 12°C control (paired t-test: $t(92) = 13.003$, $p < 2.2e\text{-}16$, Fig. 2). The mean percent change in fluorescence ranged from −96% to +120% (Fig. 3); therefore, the percent heat tolerance for the 93 gametophytes ranged from 4% to 100%. Fluorescence from the seawater + f/2 alone was negligible; the maximum value across all treatments, dates and replicates was 3.3. Mean fluorescence for the ethanol-treated gametophytes in the Control was initially 226.3 ± 22.1 and declined to 34.2 ± 9.5.

The mean percent heat tolerance of females and males selected for the Tolerant prediction group were 100.37% ± 16.19 and 74.30% ± 15.19, respectively. The mean percent heat tolerance of females and males selected for the Intolerant prediction group were 24.03% ± 1.23 and 12.32% ± 2.77, respectively.

Testing predictions with juvenile sporophytes

On average among all crosses in the Heat treatment, 87.9% of the total growth achieved between T0 and T2 was gained between T0 and T1, which was significantly greater than in

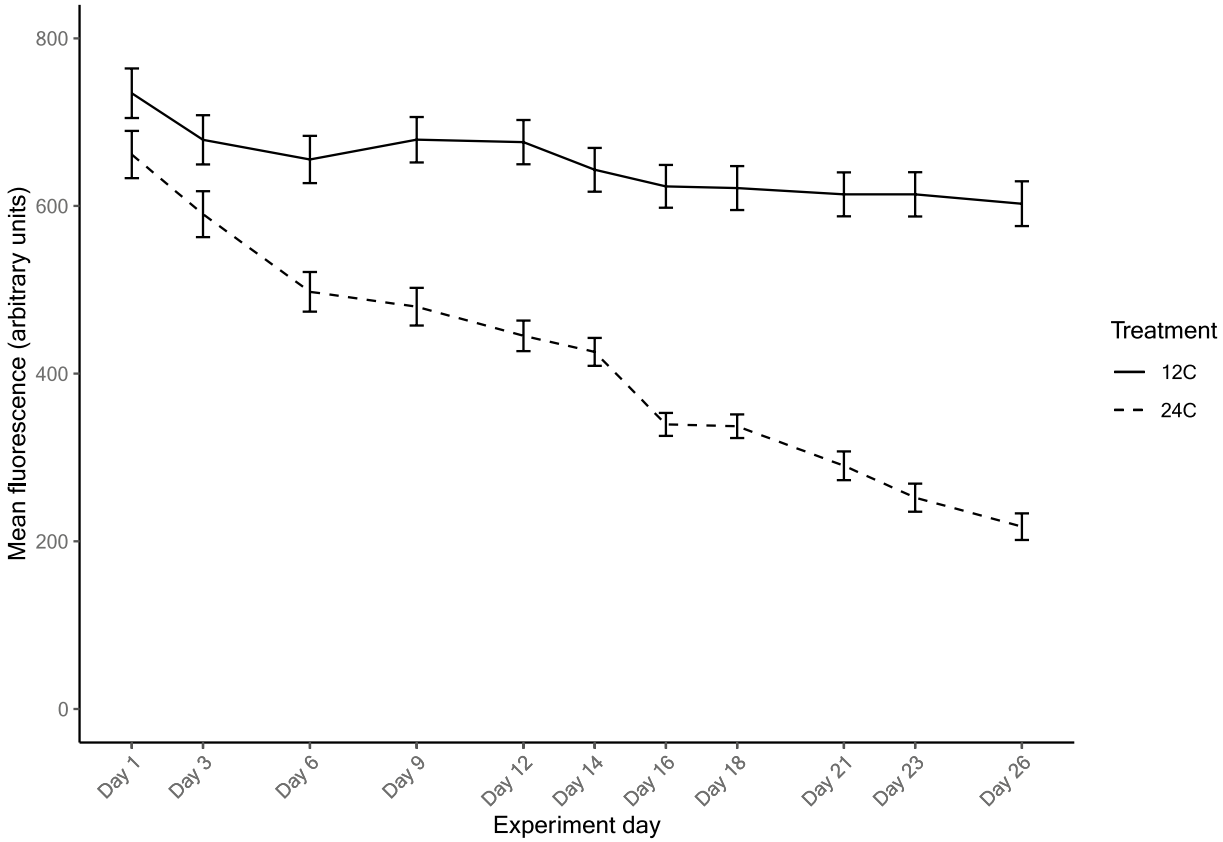

Fig. 2 Mean (± SE) fluorescence values for all 93 *Saccharina latissima* vegetative gametophytes over the experimental period in 12°C (**control; solid line**) and 24°C (**heat treatment; dashed line**) conditions. The 24 °C heat treatment underwent gradual warming from 12°C for the first 11 days, reaching 24°C on Day 12. $N = 93$ for each date for each treatment on all dates

the Control (62.8%; t-test, t(6.69) = −4.322, $p = 0.0038$; Fig. 4), and total area did not significantly change for any cross between T1 and T2 in the Heat treatment.

The percent change in total area and percent change in healthy area in juvenile sporophytes were affected by treatment, and the magnitude of the differences between treatments were affected by prediction group; total area was also affected by time (Total area: mixed model, $F_{(1,154.0)}$=10.850, $p = 0.0012$ (treatment), $F_{(1,154.0)}$=7.748, $p = 0.0061$ (treatment * prediction), $F_{(1,154.0)}$=5.078, $p = 0.025$ (time); Healthy area: mixed model, $F_{(1,154.0)}$=10.895, $p = 0.0012$ (treatment), $F_{(1,154.0)}$=12.488, $p = 0.0005$ (treatment * prediction)). Post-hoc analyses revealed that juvenile sporophytes in the Tolerant group exhibited a greater percent increase in both total area and healthy area compared to the Intolerant group in the Heat treatment (EMM, Total area: t(42.9)=2.175, $p = 0.035$; Healthy area: t(10.6)=−8.252, $p < 0.0001$; Figure 5). There were no significant differences between prediction groups in the Control in percent change in total area or percent change in healthy area. There were also no significant differences when comparing Control groups to the Tolerant sporophytes in Heat in percent change in total area or percent change in healthy area. However, Intolerant sporophytes in Heat grew less in total and healthy area compared to Tolerant sporophytes in Heat and both Tolerant and Intolerant in Control (EMM, Total area: t(56.1)=−3.462, $p = 0.001$; Healthy area: t(11.9)=−2.628, $p = 0.022$; Figure 5).

Discussion

Selective breeding for heat tolerance in kelp is critical to sustain the kelp aquaculture industry under future climate change scenarios, as well as support restoration of kelp in areas that are rapidly warming. Little is known about the relationship between gametophyte and sporophyte stress tolerance in kelps or other species with similar life histories, such as ferns (Krieg and Chambers 2022). Understanding the relationship between gametophyte and sporophyte phenotypes could bypass the need to grow out the kelp to the adult stage in order to select on phenotypes. We conducted a heat stress experiment to identify heat tolerance levels of sugar

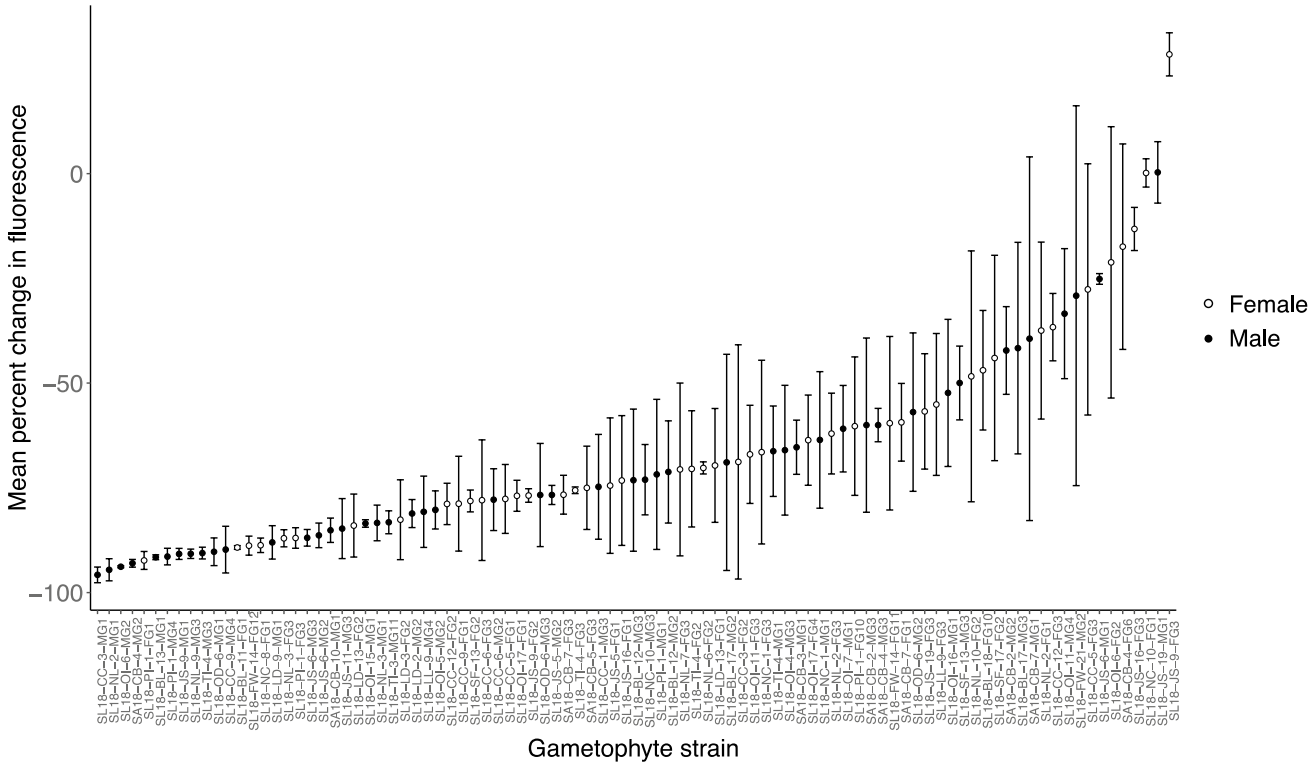

Fig. 3 Mean percent change (± SE) in fluorescence of *Saccharina latissima* vegetative gametophytes from the beginning of the experiment (12°C) through the end of the heat-stress period (Day 26; see Fig. 2) for each gametophyte strain (genotype) in the 24°C treatment. Gametophytes with values closer to 0% change indicate greater heat tolerance than those with values closer to −100%. Female gametophytes are indicated with open circles, and male gametophytes with filled circles. $N = 3$ for each gametophyte strain listed on the x-axis; gametophyte IDs follow the same format as explained in Table 2

kelp and skinny kelp gametophytes, and then tested our predictions by crossing these gametophytes and measuring the heat stress response of resulting juvenile sporophytes. Our study demonstrates that, on average, kelp sporophyte heat tolerance can be predicted based on the performance of their gametophyte parents under heat stress-testing. We developed a high-throughput method for selecting heat-tolerant gametophytes that, when crossed, produce juvenile sporophytes that grow larger and healthier at 20–22°C compared to juvenile sporophyte progeny of heat-intolerant gametophytes. The predicted Tolerant crosses not only grew better than predicted Intolerant crosses in the Heat treatment, but they also grew as well as the Tolerant and Intolerant crosses in control conditions. This has important implications for aquaculture, as it indicates that the genotypes that were able to grow better in heat did not exhibit compromised growth under cold conditions.

Maintenance of healthy tissue is critical for kelp to be able to withstand changes in temperature, since many ocean regions where kelp grow are experiencing increased average annual temperatures as well as increased variation during the year and periods of high temperatures (Smale et al. 2019; Filbee-Dexter et al. 2020). High temperatures can increase erosion of kelp tissue via holes in the cortex and central medulla, splitting of the medulla, and damage to the meristoderm (Simonson et al. 2015). Degraded tissue would slough off in the wild, which is why we measured healthy tissue in addition to total blade area. We found that predicted heat-tolerant juvenile sporophytes exhibited a higher percent of healthy area under warm conditions compared to predicted intolerant juvenile sporophytes. This higher proportion of healthy tissue could lead to greater, or at least continued, growth in the future if environmental conditions become more favorable, since the tolerant sporophytes grew just as well in cold (control) conditions. By contrast, the intolerant sporophytes would have less photosynthetic material to take advantage of these better conditions and would be unlikely to catch up to the biomass achieved by the tolerant sporophytes.

For the purposes of aquaculture, we considered the most heat-tolerant strains to be the ones that yielded the most biomass under elevated temperatures. Our Heat treatment involved gradual warming from 12°C to 22°C, so one could consider this a compressed timescale of all the temperatures the kelp would experience over the course of a farming season from autumn planting to summer harvest in nearshore

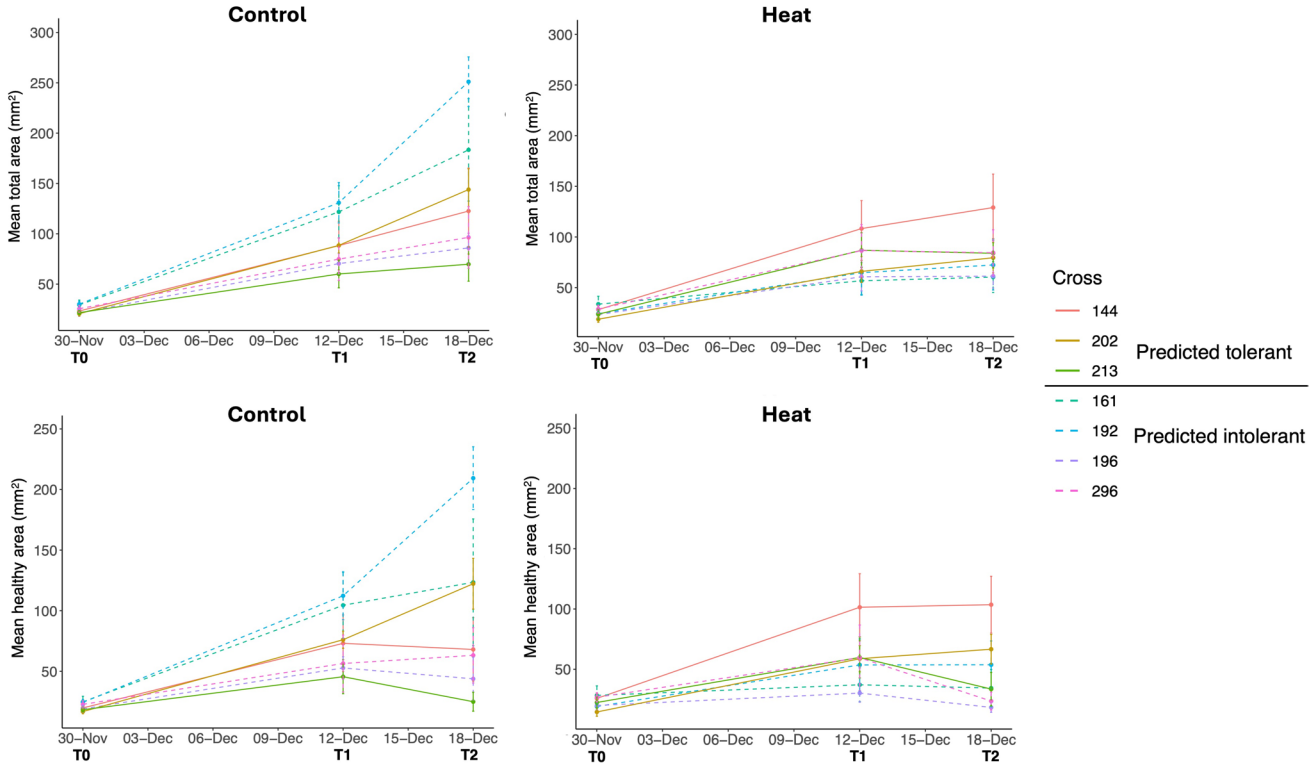

Fig. 4 Change in mean total area (± SE; top graphs) and mean healthy area (± SE; bottom graphs) for juvenile *Saccharina latissima* sporophytes in each cross in each treatment over the three timepoints, with predicted tolerant crosses as solid lines and predicted intolerant crosses as dashed lines. $N = 6$ for each cross (except for Cross 192 on the final date, where $N = 5$ due to loss of one replicate). Timepoint T0 = 30 November, T1 = 12–13 December, and T2 = 18–19 December. For simplicity, all six replicates of each cross measured at T1 and T2 are grouped together on the first date of each timepoint, 12 December and 18 December, respectively

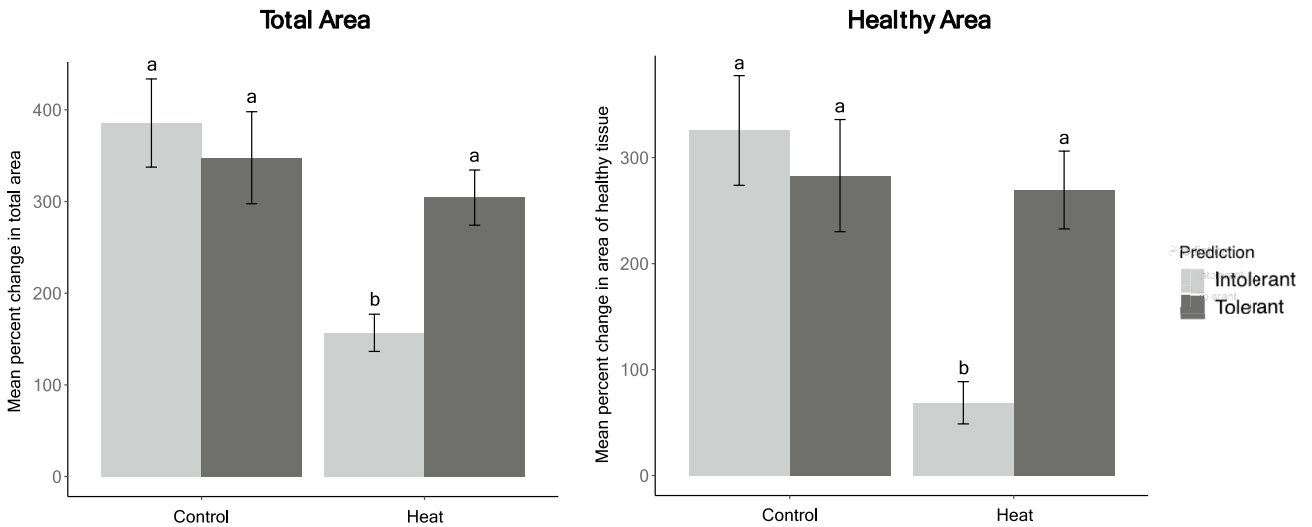

Fig. 5 Mean percent change (± SE) in total area (left) and healthy area (right) for juvenile *Saccharina latissima* sporophytes from T0 to T1 and from T0 to T2 combined in the intolerant (**light gray**) and tolerant (**dark gray**) prediction groups in the Control and Heat treatments. Groups that do not share a letter are significantly different ($p < 0.05$). $N = 36$ for Tolerant group; $N = 48$ for Intolerant group in Heat, $N = 47$ for Intolerant group in Control (due to loss of one sample after T1 measurements)

waters between Maine and New York, U.S.A. Heat tolerance in kelp experiencing increasingly warming temperatures could be achieved via two physiological strategies: 1) the kelp grows well at cooler temperatures and continues to grow equally well in warmer temperatures, resulting in high biomass at the time of harvest; 2) the kelp grows exceedingly well in cooler temperatures, so even if it does not grow as well in warmer temperatures, it still has high biomass at the time of harvest. While a kelp that has low growth in cold conditions and continues to grow slowly in warm conditions could be considered "heat-tolerant" by some definitions, this strain would likely not be profitable in aquaculture. Therefore, we chose to focus on the relative performance of different crosses under gradually increasing heat stress, rather than assessing whether a cross performed similarly in Heat compared to itself in Control, to be relevant to strain selection for farming kelp in warming waters.

Our results suggest that in order to predict sporophyte heat tolerance at a given temperature, the gametophyte parents must be stress-tested at a substantially higher temperature. In the case of this study, testing gametophytes at 24°C was effective for predicting sporophyte heat tolerance at 20–22°C, although growth even in Tolerant sporophytes tended to decline above 20°C. For kelp species, 22°C is a particularly stressful temperature; for example, it is a critical upper temperature threshold for embryonic sporophyte development of *E. radiata* in Tasmania (Mabin et al. 2013), and massive losses of *M. pyrifera* were observed in areas of Southern California and Baja California where temperatures reached at least 22°C during the 2014–2016 marine heatwave (Cavanaugh et al. 2019). Future research could address whether one gametophyte parent or the other confers more heat tolerance in the sporophyte, and how reliably the same gametophyte is able to produce heat-tolerant sporophytes when mated with various different gametophytes. With a large sample size, further work could also examine potential genomic regions related to heat tolerance.

In addition to breeding for kelp that can withstand warming temperatures at the time of harvest, kelp aquaculture would also benefit from development of juvenile sporophytes that can survive warmer temperatures for earlier planting. Sea surface temperatures on kelp farms in the Gulf of Maine are typically 13–16°C at the time of outplanting in November, but 16–20°C in September (unpublished data). If we can breed thermally robust juvenile sporophytes, then this kelp "seed" could be planted much earlier in the season, extending the growing season by up to two months and potentially resulting in larger yields. Additionally, as wild kelp beds have declined around the globe (Smale and Wernberg 2013; Filbee-Dexter et al. 2020) partially attributed to the rise in ocean temperatures, the selection of heat-tolerant gametophytes and sporophytes may become a restoration strategy to maintain these essential foundation species. There are other anthropogenic and climate change impacts on kelp life stages other than warming temperatures (see Veenhof et al. 2022), and future studies should examine multi-factorial impacts.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10811-025-03454-8.

Acknowledgements The authors would like to thank Dr. Carolyn Tepolt and Dr. Jed Goldstone for generously providing their lab space, incubators, and spectrofluorometer to conduct the experiments presented in this manuscript. The authors also thank Ruby Krasnow for conducting preliminary growth assessments of juvenile kelp sporophytes in incubators; Daniel Gossard and Morgan Anthony for assisting with image analysis of juvenile kelp sporophytes used in this study; Dr. Yaoguang Li, Dr. Jean-Luc Jannink, and Dr. Charles Yarish for offering their insights throughout the study; and Dr. Dana Morton and Dr. Pete Raimondi for their suggestions regarding statistical analyses for this manuscript.

Authors' contributions Conceptualization: Sara T. Gonzalez, Scott Lindell; Methodology: Sara T. Gonzalez, Amy Jones, David Bailey, Margaret Aydlett, Hadley Kerr, Tom W. Bell; Formal analysis and investigation: Sara T. Gonzalez, Tom W. Bell; Writing - original draft preparation: Sara T. Gonzalez; Writing - review and editing: Sara T. Gonzalez, Scott Lindell, Tom W. Bell; Funding acquisition: Scott Lindell, Sara T. Gonzalez. All authors read and approved the manuscript.

Funding This work was funded by the WHOI Carawan Postdoctoral Scholarship, the U.S. Department of Energy's ARPA-E MARINER program contract # DE-AR0000915, and the World Wildlife Fund with support from the Bezos Earth Fund and Conscience Bay Foundation.

Data availability Data are provided in supplementary information files.

Declarations

Competing interests The authors have no competing interest to declare.

Literature cited

Alsuwaiyan NA, Vranken S, Filbee-Dexter K, Cambridge M, Coleman MA, Wernberg T (2021) Genotypic variation in response to extreme events may facilitate kelp adaptation under future climates. Mar Ecol Prog Ser 672:111–121

Asthir B (2015) Protective mechanisms of heat tolerance in crop plants. J Plant Interact 10:202–110

Augyte S, Lewis L, Lin S, Neefus CD, Yarish C (2018) Speciation in the exposed intertidal zone: the case of *Saccharina angustissima* comb. nov. & stat. nov. (Laminariales, Phaeophyceae). Phycologia 57:100–112

Becheler R, Haverbeck D, Clerc C, Montecinos G, Valero M, Mansilla A, Faugeron S (2022) Variation in thermal tolerance of the giant kelp's gametophytes: suitability of habitat, population quality or local adaptation? Front Mar Sci 9:802535

Berry HD, Mumford TF, Christiaen B, Dowty P, Calloway M, Ferrier L, Grossman EE, VanArendonk NR (2021) Long-term changes in kelp forests in an inner basin of the Salish Sea. PloS ONE. 16:e0229703

Bita CE, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci 4:273

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

Bricknell IR, Birkel SD, Brawley SH, Van Kirk T, Hamlin HJ, Capistrant-Fossa K, Huguenard K, Van Walsum GP, Liu ZL, Zhu LH, Grebe G (2021) Resilience of cold water aquaculture: a review of likely scenarios as climate changes in the Gulf of Maine. Rev Aquacult 13:460–503

Buschmann AH, Camus C, Infante J, Neori A, Israel Á, Hernández-González MC, Pereda SV, Gomez-Pinchetti JL, Golberg A, Tadmor-Shalev N, Critchley AT (2017) Seaweed production: overview of the global state of exploitation, farming and emerging research activity. Eur J Phycol 52:391–406

Casado-Amezúa P, Araújo R, Bárbara I, Bermejo R, Borja Á, Díez I, Fernández C, Gorostiaga JM, Guinda X, Hernández I, Juanes JA (2019) Distributional shifts of canopy-forming seaweeds from the Atlantic coast of Southern Europe. Biodivers Conservat 28:1151–1172

Cavanaugh KC, Reed DC, Bell TW, Castorani MCN, Beas-Luna R (2019) Spatial variability in the resistance and resilience of giant kelp in Southern and Baja California to a multiyear heatwave. Front Mar Sci 6:413

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

Duarte CM, Wu J, Xiao X, Bruhn A, Krause-Jensen D (2017) Can seaweed farming play a role in climate change mitigation and adaptation? Front Mar Sci 4:100

Eullaffroy P, Vernet G (2003) The F684/F735 chlorophyll fluorescence ratio: a potential tool for rapid detection and determination of herbicide phytotoxicity in algae. Water Res 37:1983–1990

FAO (2022) The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. FAO, Rome

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

Forbord S, Matsson S, Brodahl GE, Bluhm BA, Broch OJ, Handå A, Metaxas A, Skjermo J, Steinhovden KB, Olsen Y (2020) Latitudinal, seasonal and depth-dependent variation in growth, chemical composition and biofouling of cultivated *Saccharina latissima* (Phaeophyceae) along the Norwegian coast. J Appl Phycol 32:2215–2232

Franke K, Liesner D, Heesch S, Bartsch I (2021) Looks can be deceiving: contrastingtemperature characteristics of two morphologically similar kelp species co-occurring in the Arctic. Bot Mar 64:163–175

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

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

Gauci C, Jueterbock A, Khatei A, Hoarau G, Bartsch I (2024) Thermal priming of *Saccharina latissima*: a promising strategy to improve seaweed production and restoration in future climates. Mar Ecol Prog Ser 745:59–71

Gruenburg LK, Nye J, Thorne L, Beltz B, Menz T, Chen B, Heywood E, Stepanuk J, Warren J, Flagg C (2021) New York Bight indicator report 2021. New York State Department of Environmental Conservation and State University of New York Stony Brook, New York. https://www.dec.ny.gov/docs/fish_marine_pdf/dmrsomasindicatorsii.pdf

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of Marine Invertebrate Animals. Plenum Press, New York, pp 26–60

Gulf of Maine Research Institute (2023) Gulf of maine warming update: summer. http://www.gmri.org/stories/gulf-of-maine-warming-update-summer-2023

Harden M, Kovalev M, Molano G, Yorke C, Miller R, Reed D, Alberto F, Koos DS, Lansford R, Nuzhdin S (2024) Heat stress analysis suggests a genetic basis for tolerance in *Macrocystis pyrifera* across developmental stages. Commun Biol 7:1147

Harris RJ, Bryant C, Coleman MA, Leigh A, Briceño VF, Arnold PA, Nicotra AB (2023) A novel and high-throughput approach to assess photosynthetic thermal tolerance of kelp using chlorophyll *a* fluorometry. J Phycol 59:179–192

Hemantaranjan A, Malik CP, Bhanu AN (2018) Physiology of heat stress and tolerance mechanisms—an overview. J Plant Sci Res 33:55–68

Henkel SK, Hofmann GE (2008) Differing patterns of *hsp70* gene expression in invasive and native kelp species: evidence for acclimation-induced variation. J Appl Phycol 20:915–924

Huang M, Robbins KR, Li Y, Umanzor S, Marty-Rivera M, Bailey D, Aydlett M, Schmutz J, Grimwood J, Yarish C, Lindell S (2023) Genomic selection in algae with biphasic lifecycles: a *Saccharina latissima* (sugar kelp) case study. Front Mar Sci 10:1040979

King NG, McKeown NJ, Smale DA, Wilcockson DC, Hoelters L, Groves EA, Stamp T, Moore PJ (2019) Evidence for different thermal ecotypes in range centre and trailing edge kelp populations. J Exp Mar Biol Ecol 514:10–17

Krieg CP, Chambers SM (2022) The ecology and physiology of fern gametophytes: a methodological synthesis. Applicat Plant Sci 10:e11464

Lee H, Depuydt S, Choi S, Han T, Park J (2020) Rapid toxicity assessment of six antifouling booster biocides using a microplate-based chlorophyll fluorescence in *Undaria pinnatifida* gametophytes. Ecotoxicology 29:559–570

Li Y, Umanzor S, Ng C, Huang M, Marty-Rivera M, Bailey D, Aydlett M, Jannink J, Lindell S, Yarish C (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, Fouqueau L, Valero M, Roleda MY, Pearson GA, Bischof K, Valentin K, Bartsch I (2020) Heat stress responses and population genetics of the kelp *Laminaria digitata* (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations. Ecol Evol 10:9144–9177

Liu X, Huang B (2008) Photosynthetic acclimation to high temperatures associated with heat tolerance in creeping bentgrass. J Plant Physiol 165:1947–1953

Mabin C, Gribben PE, Fischer A, Wright J (2013) Variation in the morphology, reproduction, and development of the habitat-forming

kelp *Ecklonia radiata* with changing temperature and nutrients. Mar Ecol Prog Ser 483:117–131
Mabin C, Johnson C, Wright J (2019) Family-level variation in early life-cycle traits of kelp. J Phycol 55:380–392
Mao X, Augyte S, Huang M, Hare MP, Bailey D, Umanzor S, Marty-Rivera M, Robbins KR, Yarish C, Lindell S, Jannink JL (2020) Population genetics of sugar kelp throughout the northeastern United States using genome-wide markers. Front Mar Sci 7:694
Marcum KB (1998) Cell membrane thermostability and whole-plant heat tolerance of Kentucky bluegrass. Crop Sci 38:1214–1218
Martins N, Pearson GA, Gouveia L, Tavares AI, Serrao EA, Bartsch I (2019) Hybrid vigour for thermal tolerance in hybrids between the allopatric kelps *Laminaria digitata and L. pallida* (Laminariales, Phaeophyceae) with contrasting thermal affinities. Eur J Phycol 54:548–561
Mazarrasa I, Olsen YS, Mayol E, Marbà N, Duarte CM (2014) Global unbalance in seaweed production, research effort and biotechnology markets. Biotech Adv 32:1028–1036
Mohring MB, Wernberg T, Wright JT, Connell SD, Russell BD (2014) Biogeographic variation in temperature drives performance of kelp gametophytes during warming. Mar Ecol Prog Ser 513:85–96
National Academies of Sciences, Engineering, and Medicine (2022) A Research Strategy for Ocean-based Carbon Dioxide Removal and Sequestration. The National Academies Press, Washington, DC
Nepper-Davidsen J, Andersen DT, Pedersen MF (2019) Exposure to simulated heatwave scenarios causes long-term reductions in performance in *Saccharina latissima*. Mar Ecol Prog Ser 630:25–39
Niedzwiedz S, Diehl N, Fischer P, Bischof K (2022) Seasonal and inter-annual variability in the heatwave tolerance of the kelp *Saccharina latissima* (Laminariales, Phaeophyceae). Phycol Res 70:212–222
Prasad PV, Bheemanahalli R, Jagadish SK (2017) Field crops and the fear of heat stress— opportunities, challenges and future directions. Field Crops Res 200:114–121
Quigley CT (2018) Thermal and Microbial Effects on Brown Macroalgae: Heat Acclimation and the Biodiversity of the Microbiome. Ph. D. Thesis. The University of Maine, Orono, p 246
Rajametov SN, Yang EY, Cho MC, Chae SY, Jeong HB, Chae WB (2021) Heat-tolerant hot pepper exhibits constant photosynthesis via increased transpiration rate, high proline content and fast recovery in heat stress condition. Sci Rep 11:14328
Schiel DR, Foster MS (2015) The biology and ecology of giant kelp forests. Univ of California Press, Berkeley
Schimpf NM, Liesner D, Franke K, Roleda MY, Bartsch I (2022) Microscopic stages of North Atlantic *Laminaria digitata* (Phaeophyceae) exhibit trait-dependent thermal adaptation along latitudes. Front Mar Sci 9:870792
Simonson EJ, Scheibling RE, Metaxas A (2015) Kelp in hot water: I. Warming seawater temperature induces weakening and loss of kelp tissue. Mar Ecol Prog Ser 537:89–104
Smale DA (2020) Impacts of ocean warming on kelp forest ecosystems. New Phytol 225:1447–1454
Smale DA, Wernberg T (2013) Extreme climatic event drives range contraction of a habitat-forming species. Proc R Soc B 280:20122829
Smale DA, Wernberg T, Oliver EC, Thomsen M, Harvey BP, Straub SC, Burrows MT, Alexander LV, Benthuysen JA, Donat MG, Feng M (2019) Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat Clim Change 9:306–312
Strasser FE, Barreto LM, Kaidi S, Sabour B, Serrão EA, Pearson GA, Martins N (2022) Population level variation in reproductive development and output in the golden kelp *Laminaria ochroleuca* under marine heat wave scenarios. Front Mar Sci 9:943511
Taylor-Robinson C, King NG, Foggo A, Smale DA (2024) Spatiotemporal variability in the population demography of the golden kelp, *Laminaria ochroleuca* (Phaeophyceae), at its leading range edge. Eur J Phycol 1–11
Umanzor S, Li Y, Bailey D, Augyte S, Huang M, Marty-Rivera M, Jannink JL, Yarish C, Lindell S (2021) Comparative analysis of morphometric traits of farmed sugar kelp and skinny kelp, *Saccharina* spp., strains from the Northwest Atlantic. J World Aquacult Soc 52:1059–1068
Veenhof RJ, Champion C, Dworjanyn SA, Wernberg T, Minne AJ, Layton C, Bolton JJ, Reed DC, Coleman MA (2022) Kelp gametophytes in changing oceans. Oceanogr Mar Biol Ann Rev 60:335–371
Wahid A, Gelani S, Ashraf M, Foolad MR (2007) Heat tolerance in plants: an overview. Environ Exp Bot 61:199–223
Wernberg T, Bennett S, Babcock RC, De Bettignies T, Cure K, Depczynski M, Dufois F, Fromont J, Fulton CJ, Hovey RK, Harvey ES (2016) Climate-driven regime shift of a temperate marine ecosystem. Science. 353:169–172