

1   **Effects of thermal history on reproductive success and transgenerational plasticity in the**  
2   **kelp *Laminaria pallida* (Phaeophyceae)**

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1   **Abstract**

2   As climate change threatens marine ecosystems, sessile species such as kelps are heavily  
3   impacted by rapidly changing environmental conditions. In this context, phenotypic plasticity  
4   (genotype x environment interaction) is a key acclimation mechanism to improve resilience to  
5   high temperatures. Here, we investigated the effects of thermal history on reproductive success  
6   and transgenerational plasticity in the warm-temperate kelp *Laminaria pallida*. Our results  
7   show that a warm temperature during the vegetative growth of gametophytes improved  
8   subsequent reproductive success under optimum compared to cold conditions.  
9   Transgenerational plasticity was evident; thermal tolerance of microscopic F1 sporophytes was  
10   modulated by the thermal history of the parental gametophytes, but the effects were complex  
11   varying with the physiological trait and the exposure time. Genetic lines differed in their  
12   capacity for thermal plasticity, with the direction and strength of responses more dependent on  
13   the female parental genome. This genetic variation suggests that thermal plasticity can  
14   potentially evolve under ocean warming. Our results indicated that the degree of gametophyte  
15   kinship (selfing/inbreeding versus outcrossing) does not influence the thermal response within  
16   and across generations. Overall, *L. pallida* benefits from warm conditions during the haploid  
17   (gametophyte) life stage, but milder temperatures (14-20°C) promote juvenile sporophyte  
18   performance. Our results suggest that preconditioning for reproduction and transgenerational  
19   plasticity can play an important role in *L. pallida* adaptation to warm habitats, highlighting the  
20   importance of incorporating thermal history effects in physiological studies to accurately  
21   predict the vulnerability of populations and species to future warming climates. Further  
22   understanding of kelp phenotypic plasticity will help to secure sustainable seaweed aquaculture  
23   and population restoration strategies through the establishment of priming techniques.

24

1 KEY WORDS: brown alga; gametophyte fertility; global warming; parental effects;

2 phenotypic plasticity; sporophyte formation; thermal tolerance

1    **1. INTRODUCTION**

2    Global warming is one of the greatest threats to ecosystems and biodiversity worldwide, with  
3    86% of the oceans predicted to be impacted by 2050 (e.g., Henson et al., 2017; IPCC, 2019;  
4    Kröel-Dulay et al., 2015). Species strategies to survive emerging environmental changes  
5    include distribution shifts (migration), acclimation and/or adaptation through the interplay of  
6    phenotypic plasticity and genetic variation (Donelson et al., 2019). Sessile organisms are  
7    particularly vulnerable to climate change as natural distribution shifts and adaptation via  
8    natural selection may be too slow in the face of rapidly changing conditions (Atkins & Travis,  
9    2010), posing a risk of range contraction and local extirpations. Consequently, phenotypic  
10    plasticity is critical in increasing the resilience of sessile species to changing environments  
11    (Schlichting, 1986). Phenotypic plasticity describes the capacity for phenotypic change in  
12    individuals responding to new environmental conditions without genetic changes. However,  
13    the degree of plasticity may vary between individuals and tolerant phenotypes could become  
14    fixed in the population through genetic assimilation (Donelson et al., 2019). Plasticity allows  
15    species to survive emerging stressors long enough for distribution shifts to happen or genetic  
16    adaptations to emerge (Herman & Sultan, 2011; Weigel & Colot, 2012). Phenotypic plasticity  
17    can occur both within a generation (within-generational) and across generations  
18    (transgenerational). Thus, understanding the interactions and contributions of within- and  
19    transgenerational plasticity to the phenotypic response is important for the prediction of species  
20    adaptation to changing environments (Auge et al., 2017).

21    Within-generational plasticity occurs when an individual's phenotype shifts as a direct  
22    response to environmental conditions. On the other hand, transgenerational plasticity (TGP,  
23    parental effects) is an epigenetic effect, where the parental environmental history shapes  
24    offspring phenotype (Fox et al., 2019). TGP can be adaptive if parental effects act to increase  
25    offspring fitness. Examples of adaptive TGP are present in various taxa helping to overcome

1 many different environmental stressors (Galloway & Etterson, 2007; Herman & Sultan, 2011).  
2 However, TGP can also be neutral or even maladaptive to the performance of species or  
3 populations in a changing world and has the potential to build-up across generations and  
4 increase the risk of extinction (Marshall, 2008; Sultan et al., 2009). As transgenerational  
5 plasticity must be heritable and exhibit genetic variation to evolve (Vu et al. 2015), multiple  
6 genotypes should be considered to better forecast species vulnerability and adaptive potential  
7 under future climate change. In small, closed populations with limited outcrossing  
8 opportunities, the offspring production from the mating of individuals that are genetically more  
9 closely related than random mating (inbreeding) is unavoidable (Eckert et al., 2010; Ralls et  
10 al., 2014). Accumulating evidence shows that inbreeding causes the loss of plastic responses  
11 to short-term environmental stress, contributing to reduced performance under environmental  
12 change (inbreeding depression; Keller & Waller, 2002). However, almost nothing is known  
13 about the effects of kinship on transgenerational plasticity.

14 The true kelps, brown algae of the order Laminariales are key habitat-forming species in  
15 temperate to cold-water rocky shores, supporting complex and diverse ecosystems (Hop et al.,  
16 2012; Smale, 2020), providing natural protection against coastal erosion and acting as  
17 significant long-term carbon sinks (Buchholz et al., 2012). Kelp forests also have a high  
18 economic value providing a wide range of ecosystem goods and services (e.g., Bennet et al.,  
19 2016; Blamey & Bolton 2018). As sessile species, kelps are sensitive to rapid changes in  
20 environmental conditions and many populations are currently under threat due to ocean  
21 warming, with large-scale declines in kelp abundance and geographical range shifts being  
22 reported worldwide (Krumhansl et al., 2016; Smale, 2020). Although, physiological responses  
23 to predicted ocean warming have been extensively addressed in kelps (e.g., Burdett et al., 2019;  
24 Diehl et al. 2021; Martins et al., 2017), few studies have examined the effects of environmental  
25 history within and across generations. Thermal history within the gametophyte stage was

1 reported to influence reproduction in *L. digitata* (Gauci et al., 2020; Martins et al., 2020). The  
2 potential influence of parental effects (transgenerational plasticity) in the offspring thermal  
3 phenotypic plasticity has only been recently addressed in two kelp species (*Laminaria digitata*:  
4 Gauci et al., 2020; *Ecklonia radiata*: Mabin et al., 2019).

5 Kelps are characterised by a heteromorphic life cycle alternating between microscopic stages  
6 (meiospores, gametophytes and microscopic sporophytes) and macroscopic sporophytes  
7 (Papenfuss et al., 1942). As in most kelp species life stage transitions occur with an annual  
8 rhythm controlled by seasonally recurring environmental triggers (Kain, 1989), different life  
9 stages are exposed to distinct environmental conditions. Environmental changes are likely to  
10 disturb the life cycle of kelps (Coelho et al., 2000; Martins et al., 2017). Gametophytes can  
11 remain vegetative for extended periods of time under unfavourable environmental conditions,  
12 delaying the formation of reproductive cells until conditions improve (Martins et al., 2017; tom  
13 Dieck & de Oliveira, 1993). However, how unfavourable environmental conditions  
14 experienced by gametophytes influences the offspring sporophyte performance remains  
15 unclear.

16 The split-fan kelp, *Laminaria pallida* (Greville), is mainly distributed on the South-West coast  
17 of Africa, between Danger Point in South Africa and Rocky Point in Namibia (Molloy, 1990),  
18 but it has been also observed on some islands in the Southern Ocean (e.g., Ile Saint-Paul,  
19 Papenfuss et al., 1942). In South Africa, it is one of two dominant kelp species together with  
20 *Ecklonia maxima*, while in Namibia it is the sole habitat-forming species (Rothman et al. 2017).  
21 *L. pallida* is reported as an economically and ecologically valuable kelp species in African  
22 coastal waters (Blamey & Bolton, 2018; Critchley et al., 1991). The distributional area of the  
23 species is characterized by strong upwelling and warm-temperate surface waters, from 11°C to  
24 22°C (Demarcq et al., 2003, meteonews.fr). In the northern distribution limit of the species,  
25 populations are generally sparser, more fragmented, and with lower levels of genetic diversity

1 than southern ones and are usually exposed to warmer temperatures (Assis et al., 2022;  
2 Rothman, 2015) close to the upper sporophyte survival thermal limit (22/23°C, Martins et al.,  
3 2019; tom Dieck & de Oliveira, 1993).  
4 Understanding how kelp species respond to environmental changes is of utmost importance as  
5 climate change is intensifying. Thus, this study aims to investigate the effect of thermal history  
6 within and across early life stages of a Northern range population of *L. pallida*. Gametophytes  
7 were acclimated to different thermal conditions (cold winter temperature: 8°C and warm  
8 summer temperature: 20°C) within their natural seasonal range for ~3.5 months, to evaluate  
9 whether the thermal history of gametophytes conditions the speed and success of gametophyte  
10 reproduction. Transgenerational effects were assessed by investigating whether the thermal  
11 tolerance of microscopic F1 sporophytes is affected by the thermal history of their gametophyte  
12 parents. We also examined whether thermal history effects differ among genotypes and cross  
13 types (intergametophytic selfing vs outcrossing). Deeper knowledge of thermal history effects  
14 in *L. pallida* will help to improve the management of existing populations as well as secure  
15 sustainable aquaculture and population restoration initiatives in endangered locations.

16

## 17 **2. MATERIALS AND METHODS**

### 18 **2.1. Experimental design**

19 To assess the effects of thermal history on within- and transgenerational responses, vegetative  
20 parental gametophytes were first exposed to cold (8°C) and warm (20°C) temperatures over  
21 several months (Fig. 1). Gametogenesis was then induced at an optimal temperature (14°C) and  
22 the effects of thermal history on female gametogenesis rate and reproductive success were  
23 investigated. Microscopic offspring sporophytes were reared for 16 days at a range of  
24 temperatures (8-23°C) to test the effects of parental thermal history on their thermal tolerance.

1 Several gametophyte strains from a single population were used to assess genetic variation in  
2 plasticity.

3 **2.2. Algal material**

4 Three mature sporophytes of *Laminaria pallida* were sampled from Swakopmund, Namibia  
5 (22.672 S, 14.522 E), in the Northern distributional range of the species in July 2019. There,  
6 minimum surface seawater temperatures of 12°C are recorded during winter, while it reaches  
7 22°C during the Austral summer (Demarcq et al., 2003), close to the upper survival temperature  
8 (22/23°C) of *L. pallida* sporophytes (Martins et al., 2019; tom Dieck & de Oliveira, 1993). Sori  
9 were cleaned and meiospores from each sporophyte were released separately in sterile  
10 seawater. After spore germination, separate male and female gametophyte stock cultures were  
11 established for each individual (culture numbers: 1, 3 and 6) and maintained in a vegetative  
12 state in sterile half-strength Provasoli enriched seawater (PES; Provasoli, 1968) at 12°C under  
13 3 µmol photons m<sup>-2</sup> s<sup>-1</sup> of red light and 16h:8h light:dark (L:D) cycle in a climate-controlled  
14 chamber (Fitoclima S600, Aralab, Lisbon, Portugal). Sterile artificial seawater (Tropic Marin  
15 Sea Salt, Wartenberg, Germany) with a salinity of 34 ± 1 ppm was used for maintenance and  
16 all experiments. The culture medium was changed monthly, until the beginning of the  
17 experiment (ca. 1.5y).

18

19 **2.3. Gametophyte exposure to distinct thermal conditions (cold: 8°C and warm: 20°C)**

20 Each unisexual culture of vegetative gametophytes was gently ground using a pestle and  
21 mortar, sieved and diluted to produce a stock solution of gametophyte fragments with lengths  
22 of ≤ 100 µm. From each stock solution, the volume needed to achieve densities of ~500  
23 gametophytes cm<sup>-2</sup> was added to Petri dishes (5.3 cm diameter, height 1.5 cm) containing 12  
24 ml of half-strength PES to measure the photosynthetic efficiency. Three replicate Petri dishes  
25 were used for each treatment (3 strains × 2 sexes × 2 temperatures × 3 replicates = 32 Petri

1 dishes in total). The remaining volume of each strain suspension was poured evenly into two  
2 glass tubes filled with half-strength PES. The gametophytes were allowed to recover from the  
3 mechanical stress induced by fragmentation for 14 days at 12°C, under 3  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$   
4 of red light in a 16h:8h L:D cycle. After this period, the fragmented gametophytes in the glass  
5 tubes and Petri dishes were transferred to the experimental temperatures (8°C and 20°C) and  
6 maintained under the same light conditions for ~3.5 months. The temperatures of 8°C and 20°C  
7 were chosen to reflect the annual mean minimum and maximum seawater temperature across  
8 the distribution range of *L. pallida* (Dieckmann, 1978). The culture medium was renewed  
9 weekly in the Petri dishes and every two weeks in the glass tubes. The gametophytes  
10 developing in the glass tubes were used in the following gametogenesis and sporophyte thermal  
11 tolerance experiments. Gametophyte fragmentation was performed to ensure that growth and  
12 cell division occurred under the experimental temperatures.

13

#### 14 2.3.a. Photosynthetic efficiency

15 The maximum photosynthetic yield of PSII ( $F_v/F_m$ ) was measured at the beginning and end of  
16 the long-term thermal exposure (~3.5 months) using a FluorPen FP 110 (PSI, Drásov, Czech  
17 Republic; Flash pulse: 20%, Super pulse: 70%, Actinic pulse: 10  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and used as a  
18 proxy for physiological performance. Gametophytes were dark acclimated for five minutes  
19 before the measurements. Two measurements were taken for each replicated Petri dish, and the  
20 average  $F_v/F_m$  used. Data was normalised (divided by the mean values at day 0) to account for  
21 significant differences in the initial  $F_v/F_m$  values between strains, thereby allowing comparisons  
22 over time.

23

1      **2.4. Gametophyte reproduction at optimal temperature**

2      After ~3.5 months at 8°C and 20°C (thermal history), the gametophytes were transferred to  
3      14°C by slowly increasing or decreasing the temperature at a rate of 3°C d<sup>-1</sup>. The gametophytes  
4      were allowed to acclimate at 14°C for 4 days. Gametophytes from each strain and each thermal  
5      history were then gently fragmented using a pestle and mortar, sieved and diluted in half-  
6      strength PES to produce stock solutions of gametophytes with lengths ≤ 100 µm. Densities  
7      from each strain stock solution were calculated. Crosses were obtained by combining one male  
8      and one female stock solution into Petri dishes (5.3 cm diameter, 1.5 cm height) containing 10  
9      ml of half-strength PES to achieve densities of ~600 gametophytes cm<sup>-2</sup>. Each female  
10     gametophyte strain was crossed with all three males separately, resulting in a total of 9 crosses  
11     per thermal history (Table 1). Three crosses were selfings (i.e., male and female gametophytes  
12     originating from the same sporophyte), and six were outcrosses (gametophytes derived from  
13     different sporophytes). Four replicate Petri dishes were used per treatment (9 crosses × 2  
14     thermal histories × 4 replicates = 72 Petri dishes) to monitor gametophyte growth and  
15     reproduction. Five additional Petri dishes containing four cover slips each were prepared per  
16     cross and thermal history (9 crosses × 2 thermal histories × 5 replicates = 90 Petri dishes) to  
17     check for thermal tolerance differences in the microscopic sporophyte offspring. After  
18     fragmentation, gametophytes were allowed to settle and recover for 4 days at 14°C under 3  
19     µmol photons m<sup>-2</sup> s<sup>-1</sup> of red light in a 16h:8h L:D cycle. After this period the gametophytes  
20     were transferred to 17 µmol photons m<sup>-2</sup> s<sup>-1</sup> of white light to induce gametogenesis. A  
21     temperature of 14°C was chosen as it provides favourable gametogenic conditions (Martins et  
22     al., 2019; tom Dieck & de Oliveira, 1993) and is the midpoint between thermal histories. The  
23     culture medium was changed every 11 days by the replacement of 7.5 ml of half-strength PES  
24     per Petri dish.

1      2.4.a. Gametophyte growth  
2      To assess whether thermal history influenced gametophyte growth under optimal gametogenic  
3      conditions, gametophyte area was measured on day 0 and before egg release on day 6. The area  
4      of  $\geq$  34 female and male gametophytes was measured per replicate, corresponding to 12  
5      randomly selected fields of view photographed using a Nikon D90 camera (Nikon, Tokyo,  
6      Japan) mounted on a Zeiss Observer D1 inverted microscope (Carl Zeiss MicroImaging GmbH,  
7      Göttingen, Germany) at 100 $\times$  magnification. The area of entire gametophytes present in each  
8      image was determined using ImageJ software (Schneider et al., 2012). The area measured  
9      excluded any eggs or sporophytes developed on female gametophytes. For each replicate, the  
10     average gametophyte area was calculated. Absolute growth rates (AGR) were calculated using  
11     the following formula:

$$AGR = \frac{(final\ area - initial\ area)}{experimental\ time\ (days)}$$

13  
14     2.4.b. Gametogenesis and reproductive success  
15     The relative occurrence of three ontogenetic stages in female gametophytes  
16     (vegetative/oogonia, eggs released, and sporophytes attached) was estimated every 5 days for  
17     the first 20 days and on day 28 of gametogenic conditions in a minimum of 200 female  
18     gametophytes per replicate using a Zeiss Observer D1 inverted microscope. The most advanced  
19     developmental stage was assigned for each female gametophyte. Sporophytes were considered  
20     present as soon as the first cell division was visible in the zygote. Gametogenesis rates were  
21     statistically compared between crosses and thermal histories by comparing the percentage of  
22     female fertility (i.e. gametophytes with released egg and/or attached sporophytes) on day 10,  
23     since at least one cross showed over 80% of fertile females. Reproductive success was  
24     evaluated by considering the percentage of female gametophytes with sporophytes after 28  
25     days.

1   **2.5. Juvenile offspring sporophyte exposure to thermal treatments**

2   After 23 days in reproductive conditions, microscopic sporophytes with a mean length of ~225

3   μm developed in the different crosses and thermal histories. For each thermal history, offspring

4   sporophytes from six crosses (3 selfed and 3 outcrossed) were randomly selected from the

5   initial nine prepared, ensuring that each male and each female were represented in two crosses

6   (Table 1, bold). Each of the four cover slips per Petri dish containing microscopic sporophytes

7   was transferred to a different target experimental temperature (8, 14, 20 and 23°C ± 0.5°C).

8   Target temperatures were reached by increasing or decreasing the temperature at a rate of 3°C

9   d<sup>-1</sup>. Sporophytes were exposed to each target temperature for 16 days. Temperatures of 8, 14

10   and 20°C were chosen to represent the thermal range experienced in nature across the

11   distributional range (Dieckmann, 1978, 1980) and 23°C was chosen as the upper survival

12   temperature (Martins et al., 2019; tom Dieck & de Oliveira, 1993) to assess differences between

13   thermal histories. The experiment was performed with microscopic sporophytes as

14   transgenerational effects are stronger early in life (Wilson and Reale 2006; Chang et al. 2021).

15   Four large Petri dishes (8.9 cm diameter, height 2.5 cm) containing one cover slip each and 25

16   ml of half-strength PES were used for each treatment (6 crosses × 2 thermal histories × 4

17   experimental temperatures × 4 replicates = 192 Petri dishes). Experiments were conducted in

18   temperature-controlled climatic chambers (Fitoclima S600, Aralab, Lisbon, Portugal), with 17

19   μmol photons m<sup>-2</sup> s<sup>-1</sup> of white light in a 16h:8h L:D cycle.

20

21   **2.5.a. Sporophyte density**

22   Sporophyte density was measured to assess the effect of experimental temperatures and thermal

23   histories on the survival capacity of microscopic offspring sporophytes. Sporophyte densities

24   were quantified at the beginning (day 0) and the end (day 16) of the thermal treatment. For

25   each replicate, sporophytes were counted in a minimum of 50 fields of view (Zeiss Observer

1 D1 inverted microscope; 100 $\times$  magnification). Data was normalised with respect to initial  
2 sporophyte densities between crosses, thereby allowing comparisons between crosses.

3

4 **2.5.b. Photosynthetic efficiency**

5 Maximum photosynthetic efficiency was measured on day 0 and 16 to estimate the treatment  
6 effects on the physiological performance of microscopic sporophytes. A FluorPen FP 110 (PSI,  
7 Drásov, Czech Republic) was used to measure the maximum photosynthetic yield of PSII  
8 ( $F_v/F_m$ ) as well as the response to a light curve (Light curve 1, Flash pulse: 20%, Super pulse:  
9 40%, Actinic pulse: 18  $\mu\text{mol}$ ) in each replicate. The light curve response was used to calculate  
10 the relative maximum electron transport rate (rETRmax) using the Phytotools package in R  
11 software (R Core Team, 2021; Silsbe & Malkin, 2015). Sporophytes were dark acclimated for  
12 five minutes before the measurements. Data was normalised (divided by the mean values at  
13 day 0) to account for significant differences initially between sporophytes from different  
14 crosses, allowing comparisons between crosses. Note that the photosynthetic measurements  
15 might also take into consideration the gametophyte cells that did not become reproductive.

16

17 **2.5.c. Sporophyte growth**

18 Sporophyte length was quantified at day 0 and after 8 and 16 days of thermal exposure. The  
19 length of 30 sporophytes was measured per replicate using ImageJ software (Schneider et al.  
20 2012), corresponding to 20 randomly photographed fields of view, with a maximum of two  
21 sporophytes from each picture. A Nikon D90 camera mounted on a Zeiss Observer D1 inverted  
22 microscope (100 $\times$  magnification) was used for measurements on day 0 and day 8, while on day  
23 16 a Canon Powershot A640 camera mounted on a Zeiss Axiovert 40 (Carl Zeiss MicroImaging  
24 GmbH, Göttingen, Germany; 40 $\times$  magnification) was used due to the larger sporophyte sizes.

1 The average sporophyte length was calculated for each replicated Petri dish, and the AGR was  
2 estimated according to the formula used for gametophyte growth above.

3 **2.6. Statistical Analysis**

4 Data was analysed using SPSS 27 software (IBM corp., Armonk, NY, USA) and the  
5 PERMANOVA module of Primer 6 software (Anderson, 2001; McArdle & Anderson, 2001).  
6 Data was tested for normality within groups using the Shapiro-Wilk test and homoscedasticity  
7 using Levene's test in SPSS. The normalised  $F_v/F_m$  of gametophytes was analysed under a two-  
8 factor ANOVA (fixed factors: strain and temperature). Gametophyte absolute growth rate  
9 (square root transformed), percentage of female fertility after 10 days and percentage of female  
10 gametophytes with sporophytes after 28 days were also analysed under a two-factor ANOVA  
11 (fixed factors: cross and thermal history). Sporophyte length-AGR data was analysed under a  
12 three-factor ANOVA (fixed factors: cross, thermal history and experimental temperature).  
13 Post-hoc Tukey tests with Bonferroni corrections ( $FDR \leq 0.05$ ) were conducted to determine  
14 differences between treatments when significant main effects or interactions were found.

15 Sporophyte photosynthetic data did not satisfy assumptions of normality and homoscedasticity,  
16 and thus was analysed using PERMANOVA under a three-factor design (fixed factors: cross,  
17 thermal history and experimental temperature). Analyses were performed with Euclidean  
18 distances and 9999 permutations. Post-hoc pairwise t-tests comparisons were performed to  
19 evaluate differences between treatments when significant main effect or interactions were  
20 found.

21

22 **3. RESULTS**

23 **3.1. Gametophyte fitness after long term thermal exposure**

24 The normalised maximum quantum yield of PSII ( $F_v/F_m$ ) differed significantly with  
25 temperature and strain, but there were no interactions between the two factors (Table 2, Fig.

1    2). Normalised  $F_v/F_m$  was significantly lower (ca. 1.4-fold) in gametophytes exposed to warm  
2    temperature (20°C) compared to cold temperature (8°C), irrespective of the strain. These results  
3    were corroborated by the visual appearance of the gametophytes that presented healthy brown  
4    pigmented cells at 8°C, while the gametophytes growing at 20°C had some plasmolyzed cells,  
5    indicating high stress and cell wall damage (Fig. S1). The gametophyte strains ♀1, ♀3, ♂3 and  
6    ♂6 showed lower (1.7-fold)  $F_v/F_m$  values compared to strains ♂1 and ♀6.

7

8    **3.2. Gametophyte reproduction at optimal temperature**

9    3.2.a. Gametophyte growth

10    Absolute growth rates (AGR) of gametophytes during the first 6 days of gametogenic  
11    conditions differed significantly only due to crosses (Table 3, Fig. 3). Two crosses with female  
12    strain 1 ( $\text{♀1} \times \text{♂1}$  and  $\text{♀1} \times \text{♂6}$ ) had significantly higher (1.6-fold) growth rates than  $\text{♀3} \times \text{♂6}$   
13    and  $\text{♀6} \times \text{♂3}$  crosses.

14

15    3.2.b. Female fertility and reproductive success

16    Gametogenesis was in general faster and more successful in female gametophytes with warm  
17    thermal history (WTH; 20°C) compared with cold thermal history (CTH; 8°C) (Fig. S2). At  
18    both temperatures, patterns of temporal reproductive development were similar for crosses  
19    involving the same female gametophyte strain but varied in crosses involving the same male  
20    strain. To compare initial rates of gametogenesis, the percentage of female fertility (i.e.,  
21    gametophytes with released eggs and/or attached sporophytes) was analysed after 10 days.  
22    Significant cross × thermal history interactions were detected (Table 4a, Fig. 4A). WTH  
23    gametophytes exhibited higher female fertility in all the crosses with ♀1 and ♀3 compared to  
24    CTH gametophytes. In contrast, CTH enhanced female fertility (2.0-fold) only in the cross ♀6  
25    × ♂1 compared to WTH. In the CTH gametophytes, fertility was similar in the crosses with

1      ♀1 and ♀6, while higher values were observed when both these females were crossed with ♂3  
2      than ♂1 and ♂6. The lowest percentage of fertile females occurred in the crosses ♀3 × ♂1 and  
3      ♀3 × ♂3. In WTH gametophytes, female fertility was significantly higher in crosses with ♀1  
4      compared with all other crosses.

5      For all crosses, WTH resulted in significantly higher reproductive success than CTH. The  
6      percentage of female gametophytes with sporophytes after 28 days showed a significant cross  
7      × thermal history interaction (Table 4b, Fig. 4B). In the WTH gametophytes, the relative  
8      sporophyte presence was higher in the cross ♀1 × ♂3 (97%) than in two of the crosses with  
9      ♀3 (♀3 × ♂3 and ♀3 × ♂6; mean value of 81%) and with ♀6 (♀6 × ♂1 and ♀6 × ♂6; mean  
10     value of 70%). On the other hand, the crosses ♀3 × ♂3, ♀3 × ♂6 and ♀6 × ♂3 (mean value of  
11     61%) with CTH developed a higher proportion of sporophytes than ♀1 × ♂1, ♀1 × ♂6 and ♀3  
12     × ♂1 (mean value of 39%).

13     Taken together, these female reproductive results revealed three major findings: (1) WTH  
14     history gametophytes showed greater rates of gametogenesis, with higher fertility and  
15     reproductive success; (2) Strain-specific variation was observed as gametophyte strains ♀1 and  
16     ♀3 were in general more prone to the effect of thermal history than ♀6; 3) Male gametophytes  
17     affect female gametophyte fertility, as each female strain showed different percentages of  
18     fertility when crossed with distinct male strains.

19

### 20     **3.3. Effects of parental thermal history on juvenile sporophytes**

#### 21     3.3.a. Sporophyte density

22     Sporophyte densities increased in the ♀1 crosses with CTH over the 16 days (normalised  
23     density > 1) independent of the experimental temperature, indicating ongoing gametophyte  
24     reproduction and sporophyte formation (Fig. 5). Similarly, increases in sporophyte density  
25     were also observed in the remaining crosses at 8, 14 and 20°C, regardless of the parental

1 thermal history. At the higher temperature (23°C), further maturation seems to be prevented,  
2 and the stable sporophyte densities reflect survival rather than additional development in these  
3 crosses. In the two WTH ♀1 crosses, sporophyte densities remained stable (normalised density  
4 ≈ 1) at the lowest temperatures (8 and 14°C), but decreased at higher temperatures, particularly  
5 at 23°C, indicating sporophyte mortality.

6 The normalised sporophyte density showed no significant cross × temperature × thermal  
7 history interactions, but all three two-factor interactions were significant (Table 5). Parental  
8 thermal history had no influence on the sporophyte density of both crosses with ♀6 and in the  
9 selfing ♀3 × ♂3, while higher sporophyte densities (2.3-fold) were observed in the two ♀1  
10 crosses with a CTH (Fig 5). In contrast, a WTH enhanced sporophyte density (1.2-fold) in the  
11 outcrossed ♀3 × ♂6 compared to a CTH. Overall, sporophyte densities were lower at 23°C  
12 (1.6-fold) than at all the other temperatures, while the highest densities in general were found  
13 at 14°C, irrespective of the thermal history.

14

### 15 3.3.b. Sporophyte photosynthetic responses

16 A significant cross × temperature × thermal history interaction was detected for both maximum  
17 photosynthetic yield ( $F_v/F_m$ ) and relative maximum electron transport rate (rETRmax, Table  
18 6). Overall, normalised  $F_v/F_m$  and rETRmax values were significantly higher (1.2-fold and 1.7-  
19 fold, respectively) in WTH sporophytes. This was especially prevalent for the sporophytes  
20 exposed to the highest temperature of 23°C (5 out of 6 crosses; Table S1, Figs. 6 and S3). Only  
21 in the selfed cross ♀6 × ♂6 at 8°C did CTH result in higher sporophyte  $F_v/F_m$  compared to  
22 WTH (1.2-fold).

23 In general,  $F_v/F_m$  was highest at 8°C for the CTH, but at 14°C for the WTH sporophytes (Fig.  
24 6).  $F_v/F_m$  was negatively affected by increasing experimental temperatures, being more evident  
25 in the CTH sporophytes. At the highest temperature of 23°C,  $F_v/F_m$  significantly decreased

1 compared to all the other temperatures in the sporophytes from both thermal histories (1.4-fold  
2 overall). For both thermal histories, rETRmax showed a decreasing trend at  $\geq 20^{\circ}\text{C}$  compared  
3 with the lower temperatures (Fig. S3).

4

5 **3.3.c. Sporophyte growth**

6 Over the first 8 days, the absolute growth rate (AGR) of sporophytes showed a significant  
7 interaction between all factors (cross x temperature x thermal history; Table 7a). In general,  
8 CTH sporophytes grew significantly faster (1.6-fold) than WTH sporophytes (Fig. 7, Table  
9 S2). In the sporophytes from both parental thermal histories, growth rates were generally  
10 highest at  $20^{\circ}\text{C}$ , except for WTH ♀1 crosses that grew more at  $14^{\circ}\text{C}$ . Overall, the AGRs of  
11 sporophytes were lower at  $8^{\circ}\text{C}$  (1.6-fold) and  $23^{\circ}\text{C}$  (1.5-fold) than at  $14^{\circ}\text{C}$  and  $20^{\circ}\text{C}$ , regardless  
12 of the parental thermal history. Similar growth patterns were observed in crosses sharing a  
13 female gametophyte parent and thermal history.

14 From day 8 to day 16, the AGR of sporophytes differed due to cross  $\times$  temperature and cross  
15  $\times$  thermal history interactions (Table 7b). Thermal history effects were linked to maternal  
16 gametophytes; ♀1 crosses: higher AGR (1.3-fold) were observed in the CTH sporophytes, ♀3  
17 crosses: WTH sporophytes showed higher growth rates (1.2-fold) compared to CTH  
18 sporophytes, ♀6 crosses: no significant differences on the sporophyte AGR were detected  
19 between parental thermal histories (Fig. 8, Table S2). Overall, sporophyte growth rates were  
20 highest at  $14^{\circ}\text{C}$ , significantly decreasing at  $8^{\circ}\text{C}$  (1.3-fold; in some crosses also at  $20^{\circ}\text{C}$ ) and  
21 even more at the highest temperature ( $23^{\circ}\text{C}$ , 2.9-fold).

22

23 **4. DISCUSSION**

24 Since environmental conditions are changing rapidly in marine environments, phenotypic  
25 plasticity may help to mitigate population decline by alleviating a “phenotype-environment

1 mismatch," thus buying time for evolutionary rescue. Long-term exposure to warm seawater  
2 temperature (20°C) reduced the physiological performance of gametophytes compared to cold  
3 conditions (8°C), but it promoted subsequent gametogenesis and reproductive success under  
4 optimal conditions in the year-round reproductive kelp species, *L. pallida*. This may be a  
5 product of thermal stress memory, natural seasonality in reproduction and/or of stress-induced  
6 gametogenesis. The performance of offspring sporophytes under a temperature gradient was  
7 also influenced by the thermal environment experienced by the parental gametophytes,  
8 revealing transgenerational plasticity for thermal tolerance. However, there was a surprising  
9 amount of variation in responses between crosses, physiological trait investigated and even  
10 exposure time. Genetic variation is likely responsible for the discrepancies in the reproductive  
11 output and transgenerational plasticity related to long-term thermal stress. In both reproduction  
12 and offspring thermal tolerance, the response direction and strength appear largely dependent  
13 on the female parental genome. This genetic variation constitutes a sizable advantage both for  
14 reproductive success and offspring survival and could be a target for selection as environmental  
15 conditions change. The range of responses to thermal stress varied depending on both thermal  
16 history and individuals, highlighting the importance to integrate both environmental and  
17 genetic context when assessing the effects of environmental history on kelps.

18

#### 19 **4.1. Decreased gametophyte health under warm seawater temperature**

20 Although *L. pallida* sporophytes are reproductive year-round, the peak is mainly at the end of  
21 the austral summer (March, Dieckmann, 1980), when surface temperatures are still warm (17-  
22 21°C in Swakopmund, seatemperature.org). Spores that settle on rocky substrate and develop  
23 gametophytes will most probably remain vegetative for some months until average  
24 temperatures allow gametogenesis ( $\leq 17^{\circ}\text{C}$  – May; tom Dieck and de Oliveira, 1993).  
25 Vegetative gametophytes in nature are therefore exposed to similar temperatures as the WTH

1 (20°C) used in this study. The gametophytes of *L. pallida* maintained under WTH for ~3.5  
2 months showed reduced physiological health (low photosynthetic efficiency and presence of  
3 plasmolyzed cells indicating stress) compared to gametophytes growing under CTH (8°C).  
4 Although 20°C is within the thermal range of *L. pallida*, the upper survival temperature of  
5 gametophytes has been shown to decrease with exposure time, surviving 27°C for 1 day, 25°C  
6 for 2 weeks, while this limit decreases to 23°C after 8 weeks (tom Dieck, 1993). Our results  
7 suggest that the gametophytes exposed to ~20°C *in situ* may already experience sublethal  
8 conditions affecting physiological health. Possible increases in water temperatures due to  
9 climate change may therefore subject gametophytes to sublethal conditions with potential  
10 impacts on reproductive output and/or success.

11 In rarer cases of spore release during colder months, gametophytes may become reproductive  
12 (8°C; Martins et al. 2019) or settle in a vegetative state below the canopy under dark conditions  
13 until irradiance is sufficient to trigger gametogenesis. Our results suggest that gametophytes  
14 would remain healthy during prolonged exposure to low temperatures.

15

#### 16 **4.2. Warm thermal history promotes gametogenesis and female reproductive success**

17 We found that gametophyte thermal history impacts female gametophyte reproduction in *L.*  
18 *pallida*. Although the development under warm seawater temperature (20°C) reduced the  
19 gametophyte physiological performance, it promoted the speed of gametogenesis and the  
20 reproductive success under optimal conditions. Similarly, in the kelp *Alaria esculenta* faster  
21 gametogenesis in WTH (22°C) gametophytes was suggested to explain enhanced growth in  
22 sporophytes with a parental WTH compared to CTH (12°C) (Quigley et al., 2018). However,  
23 these results contrast with previous studies on cold-adapted kelp species, where WTH led to  
24 decreases in the fertility of gametophytes (*L. digitata*: Martins et al., 2020; Gauci et al. 2020).  
25 Together, these results suggest that in kelps thermal stress memory might affect the timing of

1 gametogenesis and reproductive success, but that responses to cold or heat stress memory may  
2 be species-specific.

3 The WTH promotion of *L. pallida* gametogenesis and sporophyte formation is consistent with  
4 its seasonal reproductive pattern in the native environment. Spores release at the end of the  
5 summer (Rothman et al., 2015) suggests that gametophyte development occurs seasonally  
6 under high summer/autumn seawater temperatures, while sporophyte formation occurs during  
7 lower winter temperatures (Dieckmann, 1978). Reproduction in Laminariales is known to be  
8 associated with seasonal environmental cues, including changing seawater temperature,  
9 daylength or irradiance (de Bettignies et al. 2018; Martins et al., 2017; Kain 1989). The warm  
10 thermal environment to which gametophytes were exposed may have acted as a seasonal  
11 thermal cue, preconditioning gametophytes for reproduction when cooler conditions followed.

12 In other brown algae, like fucoids, gametogenesis initiates in response to short days (Bäck et  
13 al., 1991; Berger et al., 2001), whereas water movements and lunar or tidal cycles are key  
14 proximal signals in gamete release (Monteiro et al. 2016; Pearson et al., 1998; Pearson &  
15 Serrão, 2006; Serrao et al., 1996). In some Arctic kelp species, gametogenesis occurs only  
16 under short days (<8 h light) and low seawater temperatures (Wiencke & tom Dieck, 1989).

17 The warm-temperate kelp *Ecklonia radiata* produces the healthiest gametophytes when days  
18 are long to promote germination and growth, but gametogenesis and sporophyte production  
19 occurs later when daylengths are shorter and water temperature declines (Mohring et al., 2013).

20 In the transition from vegetative growth to gametogenesis, the thermal history of gametophytes  
21 may affect the rate or extent to which ribosome, transcription and translation related pathways  
22 are triggered, impacting gametogenesis and reproductive success (Pearson et al., 2019).

23 There is extensive evidence of heat-stress induced flowering in plants (reviewed by Takeno &  
24 Raines, 2016) and seagrasses (Blok et al., 2018; Marín-Guirao et al., 2019; Ruiz et al., 2018),  
25 and is interpreted as an ultimate stress adaptation. Similarly, heat stress enhanced the

1 production of new gametophytic thalli accelerating the asexual reproduction in the red alga  
2 *Pyropia yezoensis* (Suda & Mikami, 2020). Such stress-related effects may add to the seasonal  
3 conditioning in promoting reproduction in WTH gametophytes of *L. pallida*.

4

5 **4.3 Female gametophyte fertility is shaped by the male strain**

6 It is known for long that kelp fertilization is facilitated by the universal pheromone lamoxirene  
7 secreted by the female gametophyte when eggs are produced. Lamoxirene triggers  
8 spermatozoid release from antheridia and their attraction to the eggs (Lüning and Muller 1978).  
9 Although, no pheromone production or chemical signalling by male gametophytes has been  
10 demonstrated in kelps, recent evidence shows the influence of male gametophytes on female  
11 fertility. Martins et al. (2019) discovered that female gametogenesis in *L. digitata* and *L. pallida*  
12 was promoted when male gametophytes were present. Furthermore, female fecundity and  
13 fertility was also shown to be dependent on male presence, identity and kinship in the giant  
14 kelp, *Macrocystis pyrifera* (Camus et al. 2021). Similarly, in our study the female  
15 gametogenesis development was shaped by the male gametophyte strain. However, no clear  
16 pattern was found, i.e., the same male did not lead to the same reproductive output independent  
17 of the female strain. Taken together, these findings support the existence of a complex  
18 bidirectional mechanism of chemical communication between female and male kelp  
19 gametophytes that deserves further attention.

20

21 **4.4. Transgenerational effects on the thermal tolerance of microscopic sporophytes**

22 *L. pallida* gametophytes from northern populations most probably develop and grow *in situ*  
23 under warm summer temperatures, whereas sporophyte recruitment happens in early winter  
24 and juvenile sporophytes grow under mild temperatures (12-16°C; Dieckmann, 1978). In our  
25 study, *L. pallida* microscopic sporophytes displayed transgenerational thermal plasticity.

1 Sporophyte from WTH parental conditions showed higher photosynthetic efficiency,  
2 particularly at moderate to high temperatures (14-23°C) compared to CTH. Several studies  
3 have demonstrated that prior exposure to high temperatures can enhance tolerance in marine  
4 macrophytes when exposed to thermal stress (heat-stress memory; *Zostera muelleri* and  
5 *Posidonia australis*: Nguyen et al., 2020; *Bangia fuscopurpurea*: Kishimoto et al., 2019; *Fucus*  
6 *vesiculosus*: Li and Brawley, 2004). Parental thermal history shaped the thermal performance  
7 curve of photosynthetic efficiency in *L. pallida* offspring. The temperature at which the  
8 sporophytes show the highest  $Fv/Fm$  values shifted from 8°C in CTH sporophytes to 14°C in  
9 sporophytes with a parental WTH. This is in line with earlier studies in various organisms  
10 showing that prior environmental history can influence thermal performance, with higher  
11 acclimation temperatures resulting in upwards thermal optima shifts (Samuels et al. 2021;  
12 Klepsat et al. 2020; Seebacher et al., 2015; Sendall et al. 2015). Thus, our study confirms that  
13 the temperature at which maximum performance occurs can respond to changes in  
14 environmental conditions.

15 Although studies on thermal plasticity across generations have recently increased for marine  
16 organisms (Gauci et al. 2020; McRae et al., 2021; Shama et al., 2016), the temporal effects on  
17 offspring performance are still unclear. In our study, sporophyte growth responses varied over  
18 exposure time to a range of temperatures; sporophytes from CTH grew significantly more than  
19 WTH during the first 8 days, particularly at mild to warm temperatures (14-23°C). This may  
20 represent a potential “silver-spoon” parental effect (Baker et al., 2019; Germain et al., 2019),  
21 which describes a physiological advantage for individuals whose parents had access to  
22 abundant resources. Similarly, in the cold-temperate kelp *L. digitata* the growth of early  
23 sporophytes at extreme temperatures (0°C and 20°C) was improved by a cold parental thermal  
24 history (5°C; Gauci et al. 2020). Interestingly, the “silver spoon” parental effect disappeared  
25 over time (from day 8 to day 16) and thermal plasticity dependent on the maternal line become

1 evident. In the offspring from maternal line ♀1 growth was enhanced by a parental CTH, while  
2 the WTH sporophytes from maternal line ♀3 grew more than those from CTH. On the other  
3 hand, parental thermal history had no influence on the growth of the offspring from maternal  
4 line ♀6. These results suggest a more complex picture of the effects of thermal history on the  
5 offspring performance of this warm temperate kelp species that are dependent of the  
6 temperature exposure duration in the offspring.

7 Parental WTH was expected to enhance the growth of the offspring sporophytes as was  
8 observed for the photosynthetic efficiency, however sporophyte growth did not conform to our  
9 predictions. This might be associated with a possible fecundity-growth trade-off, where WTH  
10 sporophytes might have less energy available to invest in growth due to the successful  
11 reproduction during the parental stage and thus increases photosynthesis efficiency, while CTH  
12 sporophytes might still have parental resources available for growth not requiring increasing  
13 the photosynthetic performance. Measurement of other stress-related traits (e.g., antioxidant  
14 enzymes) at sublethal temperatures may help elucidate the observed responses. Together, the  
15 results show that the thermal environment experienced by the parental gametophytes influences  
16 the response plasticity of offspring sporophytes, however the effects are complex, varying  
17 according to the physiological traits investigated, exposure time, and genotype, thus further  
18 studies are needed to provide more insight into this understudied topic.

19

20 **4.5. Genetic variation in reproductive success and transgenerational plasticity: via female  
21 or maternal effects**

22 Genetic variation in reproductive success and transgenerational plasticity for thermal tolerance  
23 was demonstrated for *L. pallida*. The response of gametophytes to thermal history was  
24 dependent on the female genetics. The WTH crosses with ♀1 and ♀3 showed enhanced fertility  
25 and offspring output. Crosses with ♀6 also showed increased offspring output after 28 days,

1 although there was no clear difference between thermal histories after 10 days. Similarly, the  
2 effect intensity differed between crosses with ♀1 and ♀3 (stronger in ♀1 crosses with respect  
3 to reproductive success between WTH and CTH gametophytes). These variations in  
4 reproductive response within a generation may be linked to the aptitude to form and retain  
5 epigenetic markers, e.g., DNA methylation or histone modification. Genetic variation in  
6 plasticity has been extensively described in plants (Sultan et al., 2009; Vu et al., 2015) and was  
7 recently shown in kelp species (Alsuwaiyan et al., 2021; Mabin et al., 2019; Liesner et al.,  
8 2020). Alsuwaiyan et al. (2021) found that haploid and diploid life stages of *Ecklonia radiata*  
9 genotypes differed in their susceptibility to thermal stress. Similarly, in *Laminaria digitata*  
10 variation in thermal plasticity was reported among genetic lines within a North Sea population  
11 (Liesner et al., 2020).

12 Similarly, the direction and strength of the transgenerational plasticity of offspring sporophytes  
13 was affected by the maternal genome or epigenome. Sporophyte mortality was only observed  
14 in ♀1 crosses with a parental WTH when exposed to the highest temperature (23°C). Moreover,  
15 different transgenerational effects on sporophyte growth rates (from day 8 to day 16 of thermal  
16 exposure) were observed for each maternal line, suggesting a strong maternal effect on the  
17 aptitude for transgenerational plasticity. These results suggest that epigenetic mechanisms are  
18 retained more during maternal than paternal gametogenesis. Maternal effects on  
19 transgenerational plasticity have been observed in a variety of taxa (e.g., Galloway & Etterson,  
20 2007; Marshall, 2008; Shama et al., 2014) and are considered the norm, as females have more  
21 ways to impact offspring development (e.g., egg provisioning, transferable maternal material  
22 such as mRNA and heritable heat-shock proteins). Evidence of maternal effects on offspring  
23 performance also exist in Laminariales (Mabin et al., 2019; Martins et al., 2019; tom Dieck &  
24 de Oliveira, 1993; Zhang et al., 2011). For example, in *E. radiata* it was proposed that maternal  
25 effects were associated with the gametophyte morphological variation to different temperature

1 and light levels (Mabin et al. 2019). Moreover, thermal responses of interspecific hybrids  
2 between *L. digitata* and *L. pallida* revealed that female parents are more important in  
3 determining the offspring phenotype than male parents (Martins et al., 2019). Similarly, the  
4 female parent was responsible for the sensitivity to low temperature in hybrids between *L.*  
5 *pallida* and *L. abyssalis* (tom Dieck & de Oliveira, 1993).

6 The genetic variation of transgenerational plasticity is double-edged as the genetic component  
7 of plasticity offers a target for selection, potentially increasing the resilience and fitness of  
8 genetically diverse populations (Chevin et al., 2010; Munday et al., 2017, 2019), but such  
9 selection is likely to lead to a loss of genetic variation. In isolated, sparse populations like that  
10 studied here, this might lead to problematic inbreeding rates.

11 Our results highlight the importance of considering the degree of genetic variation that is  
12 involved in kelp transgenerational plasticity. Studies elucidating the molecular mechanisms  
13 contributing to differential responses under common environmental conditions will be an  
14 important next step.

15

#### 16 **4.6. Plasticity is not influenced by gametophyte kinship**

17 Kelp species are capable of intergametophytic selfing, i.e., mating between a female and a male  
18 gametophyte derived from the same diploid sporophyte (Raimondi et al., 2004; Carney et al.,  
19 2013; Itou et al., 2019), potentially resulting in inbreeding depression. Selfing leads to a variety  
20 of negative fitness effects in *Macrocystis pyrifera* (Raimondi et al., 2004; San Miguel, 2017;  
21 Camus et al., 2021). In contrast, we found no influence of selfing vs outcrossing on female  
22 reproductive success or thermal tolerance of offspring sporophytes in *L. pallida*, regardless of  
23 the thermal history. However, our study was confined to one relatively isolated population with  
24 low genetic diversity (Assis et al., 2022), and the costs of selfing may vary between  
25 populations.

1 Remote and patchy populations of several kelp species tend to suffer less from inbreeding than  
2 dense central forest networks (Barner et al., 2011; Camus et al., 2018, 2021). Populations of *L.*  
3 *pallida* from Northern Namibia, including the one used in this work, are much less diverse than  
4 those in the southern range (Assis et al. 2022), thus it is likely that outcrossing offers only  
5 slightly greater heterozygosity than selfing, reducing the capacity to detect the effects of  
6 inbreeding depression against a low diversity background. The absence of inbreeding  
7 depression may be also attributed to genetic ‘purging’. The accumulated deleterious alleles in  
8 populations that suffered reductions in size may be purged through natural selection (Barrett &  
9 Charlesworth, 1991; Byers & Waller, 1999). To better understand potential relationships  
10 between mating systems and expression of phenotypic plasticity, it would be interesting to  
11 explore whether the absence of selfing on fitness and phenotypic plasticity differs among *L.*  
12 *pallida* populations with different levels of inbreeding.

13 Together, our results suggest that thermal plasticity play an important role in the response of  
14 this Northern range population of *L. pallida* to environmental change, contributing to their  
15 successful persistence and adaptation. However, it also reveals that these responses are  
16 complex, depending on thermal history, maternal genetics, time of exposure to thermal stress  
17 and physiological traits, and such dependency needs to be considered when predicting  
18 organismal and/or population responses to a changing world. We are far from fully  
19 understanding the intricacies and complexity of transgenerational plasticity responses of kelps  
20 to environmental stressors. Thus, future research should investigate the extent at which  
21 phenotypic plasticity could buffer kelp populations with multiple environmental stressors and  
22 their stability across multiple generations.

23

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- 20

1 **Table 1.** Crosses used to evaluate gametophyte reproduction (3 selfed and 6 outcrossed). The  
 2 crosses used to evaluate thermal plasticity of microscopic F1 sporophytes are highlighted in  
 3 bold (3 selfed and 3 outcrossed).

<b>Strain</b>	$\text{\textcircled{f}}\text{1}$	$\text{\textcircled{f}}\text{3}$	$\text{\textcircled{f}}\text{6}$
$\text{\textcircled{g}}\text{1}$	<b>Selfed</b>	<b>Outcrossed</b>	Outcrossed
$\text{\textcircled{g}}\text{3}$	Outcrossed	<b>Selfed</b>	<b>Outcrossed</b>
$\text{\textcircled{g}}\text{6}$	<b>Outcrossed</b>	Outcrossed	<b>Selfed</b>

4  
 5 **Table 2.** ANOVA for the effects of strain and temperature on the  $F_v/F_m$  of *Laminaria pallida*  
 6 gametophytes. The post-hoc results are presented in Fig. 2

<b>Factor</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Strain	5	1.56	0.31	16.33	<b>&lt;0.001</b>
Temperature	1	0.55	0.55	28.93	<b>&lt;0.001</b>
Strain $\times$ Temperature	5	0.10	0.02	1.01	0.434
Residual	24	0.46	0.02		

7 Significant interactions or main effects are highlighted in bold. df: degrees of freedom; SS:  
 8 sum of squares; MS: mean sum of squares.  
 9

10 **Table 3.** ANOVA for the effects of cross and thermal history (8 and 20°C) on the absolute  
 11 growth rate for gametophyte area of *Laminaria pallida* after 6 days in gametogenic conditions.  
 12 The post-hoc results are presented in Fig. 3.

<b>Factor</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Cross	8	309.2	38.63	4.66	<b>&lt;0.001</b>

Thermal history	1	24.43	24.43	2.95	0.092
Cross × Thermal history	8	99.78	12.47	1.51	0.177
Residual	53	438.93	8.28		

1 Significant interactions or main effects are highlighted in bold. df: degrees of freedom; SS:  
 2 sum of squares; MS: mean sum of squares.

3

4 **Table 4.** ANOVA for the effects of cross and thermal history (8 and 20°C) on the percentage  
 5 of reproductive female gametophytes after 10 days (a) and percentage of female gametophytes  
 6 with sporophytes after 28 days (b) in gametogenic conditions. The post-hoc results are  
 7 presented in Fig. 4.

Factor	df	SS	MS	F	P
<hr/>					
(a) % Female gametophyte fertility					
at day 10					
Cross	8	16292.94	2036.62	64.98	<0.001
Thermal History	1	10268.89	10268.89	327.62	<0.001
Cross × Thermal history	8	13415.90	1676.99	53.50	<0.001
Residual	53	1661.25	31.34		
(b) % Female gametophytes with					
sporophytes at day 28					
Cross	8	2105.45	263.18	8.40	<0.001
Thermal history	1	19332.97	19332.97	617.04	<0.001
Cross × Thermal history	8	3488.85	436.11	13.92	<0.001
Residual	53	1669.59	31.33		

8 Significant interactions or main effects are highlighted in bold. df: degrees of freedom; SS:  
 9 sum of squares; MS: mean sum of squares.

1

2 **Table 5.** ANOVA for the effects of cross, temperature (8, 14, 20 and 23°C) and thermal history  
 3 on the normalized density of microscopic sporophytes of *Laminaria pallida* after 16 days. The  
 4 post-hoc results are presented in Fig. 5.

<b>Factor</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Cross	5	2.62	0.52	6.55	<b>&lt;0.001</b>
Temperature	3	11.57	3.86	48.20	<b>&lt;0.001</b>
Thermal history	1	2.63	2.63	32.90	<b>&lt;0.001</b>
Cross × Temperature	15	4.74	0.32	3.95	<b>&lt;0.001</b>
Cross × Thermal history	5	14.74	2.95	36.84	<b>&lt;0.001</b>
Temperature × Thermal history	3	1.99	0.67	1.42	<b>&lt;0.001</b>
Cross × Temperature × Thermal history	15	1.70	0.11		0.15
Residual	144	11.52	0.08		

5 Significant interactions or main effects are highlighted in bold. df: degrees of freedom; SS:  
 6 sum of squares; MS: mean sum of squares.

7

8 **Table 6.** PERMANOVA for the effects of cross, temperature (8, 14, 20 and 23°C) and thermal  
 9 history on the normalised photosynthetic efficiency of *Laminaria pallida* sporophytes after 16  
 10 days. The post-hoc results are presented in Figs. 6 and S3 and Table S1.

<b>Factor</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>Pseudo - F</b>	<b>P(perm)</b>
(a) Normalised $F_v/F_m$					
Cross	5	3.09	0.62	38.63	<b>0.0001</b>
Temperature	3	4.53	1.51	94.49	<b>0.0001</b>
Thermal history	1	1.20	1.20	74.90	<b>0.0001</b>

Cross × Temperature	15	0.38	0.03	1.57	0.0901
Cross × Thermal history	5	0.13	0.03	1.66	0.1453
Temperature × Thermal history	3	0.46	0.15	9.50	<b>0.0002</b>
Cross × Temperature × Thermal history	15	0.63	0.04	2.64	<b>0.0016</b>
Residual	144	2.30	0.02		

(a) Normalised rETRmax					
Cross	5	44.89	8.98	44.45	<b>0.0001</b>
Temperature	3	33.35	11.12	55.04	<b>0.0001</b>
Thermal history	1	20.81	20.81	103.03	<b>0.0001</b>
Cross × Temperature	15	12.16	0.81	4.01	<b>0.0001</b>
Cross × Thermal history	5	13.77	2.75	13.64	<b>0.0001</b>
Temperature × Thermal history	3	0.93	0.31	1.55	0.2049
Cross × Temperature × Thermal history	15	5.76	0.38	1.90	<b>0.0264</b>
Residual	144	29.08	0.20		

1      Significant interactions or main effects are highlighted in bold. df: degrees of freedom; SS:

2      sum of squares; MS: mean sum of squares.

3

4      **Table 7.** ANOVA for the effects of cross, temperature (8, 14, 20 and 23°C) and thermal history

5      on the absolute growth rate of sporophytes of *Laminaria pallida* after two time periods, (a) 0 -

6      8 days and (b) 8 - 16 days. The post-hoc results are presented in Figs. 7 and 8 and Table S2.

Factor	df	SS	MS	F	P
(a) Absolute growth rate, d0-d8					
Cross	5	7306.75	1461.35	59.26	<0.001
Temperature	3	5131.29	1710.43	69.36	<0.001

Thermal history	1	6509.18	6509.18	263.96	<b>&lt;0.001</b>
Cross × Temperature	15	624.48	41.63	1.69	0.059
Cross × Thermal history	5	1525.25	305.05	12.37	<b>&lt;0.001</b>
Temperature × Thermal history	3	142.41	47.47	1.93	0.128
Cross x Temperature × Thermal history	15	930.61	62.04	2.52	<b>0.002</b>
Residual	144	3550.99	24.66		

(b) Absolute growth rate, d8-d16

Cross	5	19822.20	3964.44	23.00	<b>&lt;0.001</b>
Temperature	3	145758.89	48586.30	281.90	<b>&lt;0.001</b>
Thermal history	1	730.78	730.78	4.24	0.041
Cross × Temperature	15	14662.34	977.49	5.67	<b>&lt;0.001</b>
Cross × Thermal history	5	11092.36	2218.47	12.87	<b>&lt;0.001</b>
Temperature × Thermal history	3	613.22	204.40	1.19	0.317
Cross x Temperature × Thermal history	15	3725.04	248.34	1.44	0.136
Residual	144	24818.65	172.35		

1 Significant interactions or main effects are highlighted in bold. df: degrees of freedom; SS:

2 sum of squares; MS: mean sum of squares.

3

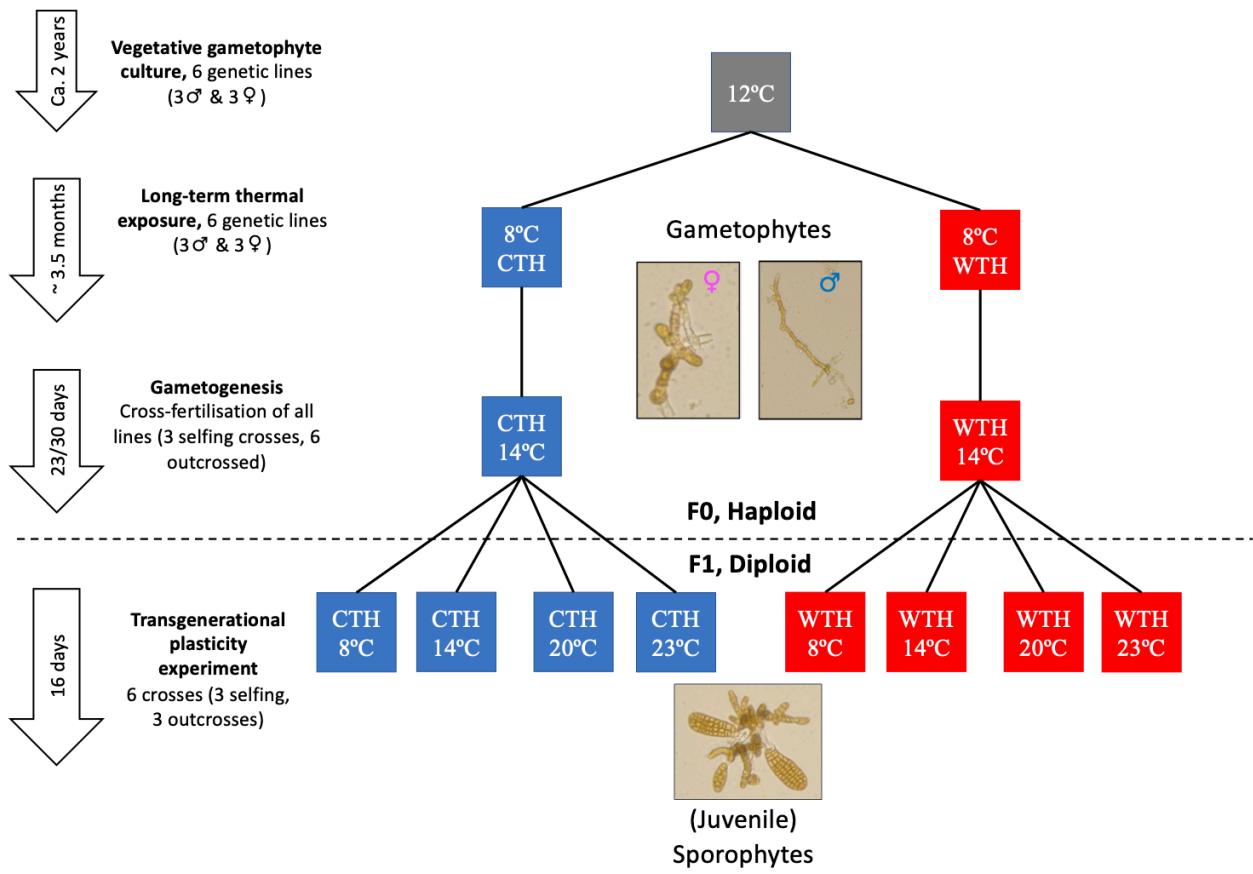
1   **Table S1.** Summary of the number of significant differences between thermal histories (8°C:  
 2   CTH and 20°C: WTH) per experimental temperature (8, 14, 20 and 23°C) in the photosynthetic  
 3   parameters of *Laminaria pallida* sporophytes.

<b>Photosynthetic parameter</b>	<b>F<sub>v</sub>/F<sub>m</sub></b>	<b>rETRmax</b>			
<b>Nº differences</b>		CTH > WTH	WTH > CTH	CTH > WTH	WTH > CTH
8°C		1	1	0	3
14°C		0	4	0	3
20°C		0	3	0	2
23°C		0	5	0	4

4  
 5  
 6   **Table S2.** Summary of the number of the significant differences between thermal histories  
 7   (8°C: CTH and 20°C: WTH) per experimental temperature (8, 14, 20 and 23°C) in the absolute  
 8   growth rate of *Laminaria pallida* sporophytes.

<b>Time period</b>	<b>0-8 days</b>		<b>8-16 days</b>	
<b>Nº differences</b>	CTH > WTH	WTH > CTH	CTH > WTH	WTH > CTH
8°C	3	0	2	2
14°C	4	0	2	2
20°C	5	0	2	2
23°C	4	0	2	2

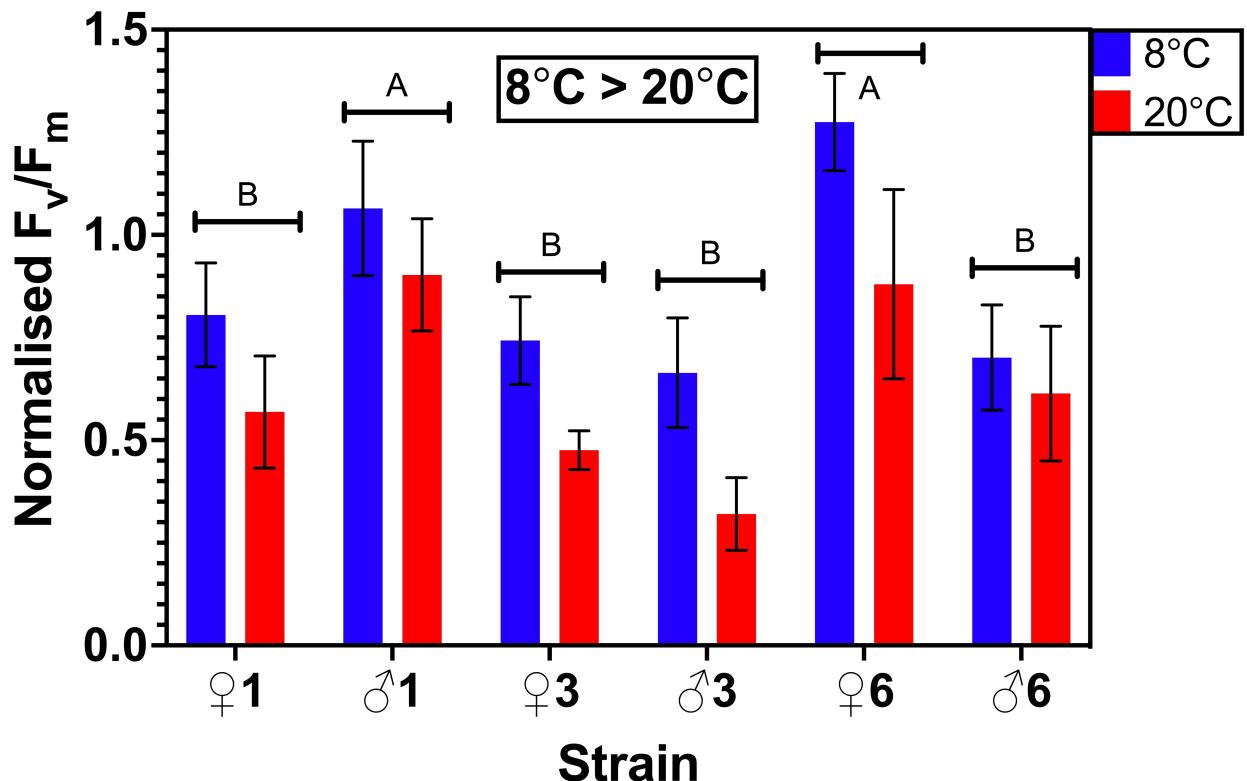
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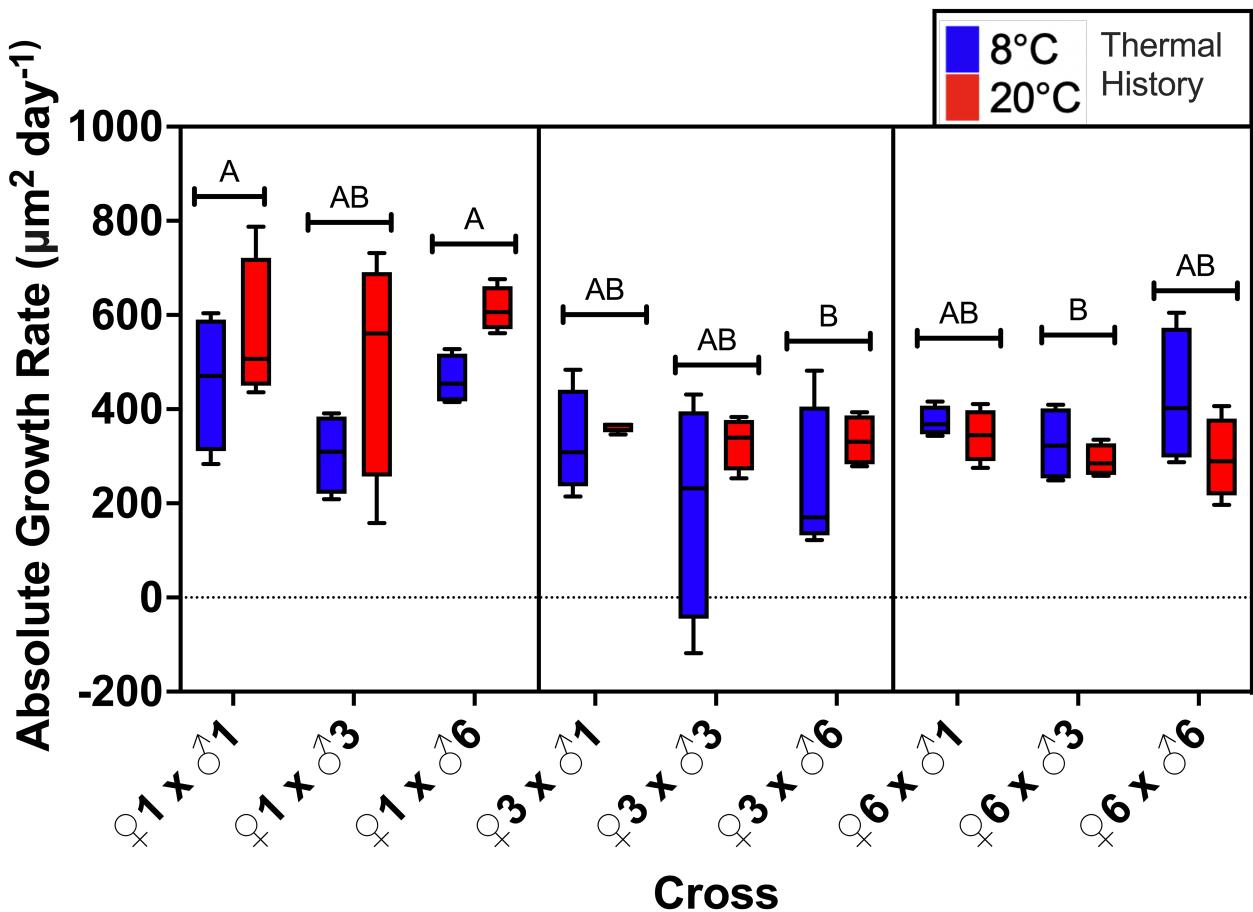
1

2 **Figure 1: Experimental design to test for within- and transgenerational plasticity of**  
 3 ***Laminaria pallida*.** Male and female gametophytes from three sporophytes were isolated and  
 4 grown vegetatively for 1.5 years and subsequently exposed to 8°C (CTH = Cold Thermal  
 5 History) and 20°C (WTH = Warm Thermal History) for 3.5 months. All genetic lines from both  
 6 thermal histories were crossed producing nine crosses, three selfed and six outcrossed and  
 7 exposed to optimal gametogenic conditions (14°C). Following sporophyte formation, six  
 8 crosses were selected and transferred to four experimental temperatures (8, 14, 20, 23°C) to  
 9 test for thermal plasticity (16 days). The dashed line shows the transition between haploid ( $n =$   
 10 1) and diploid ( $n = 2$ ) stages and between generations (F0, F1).

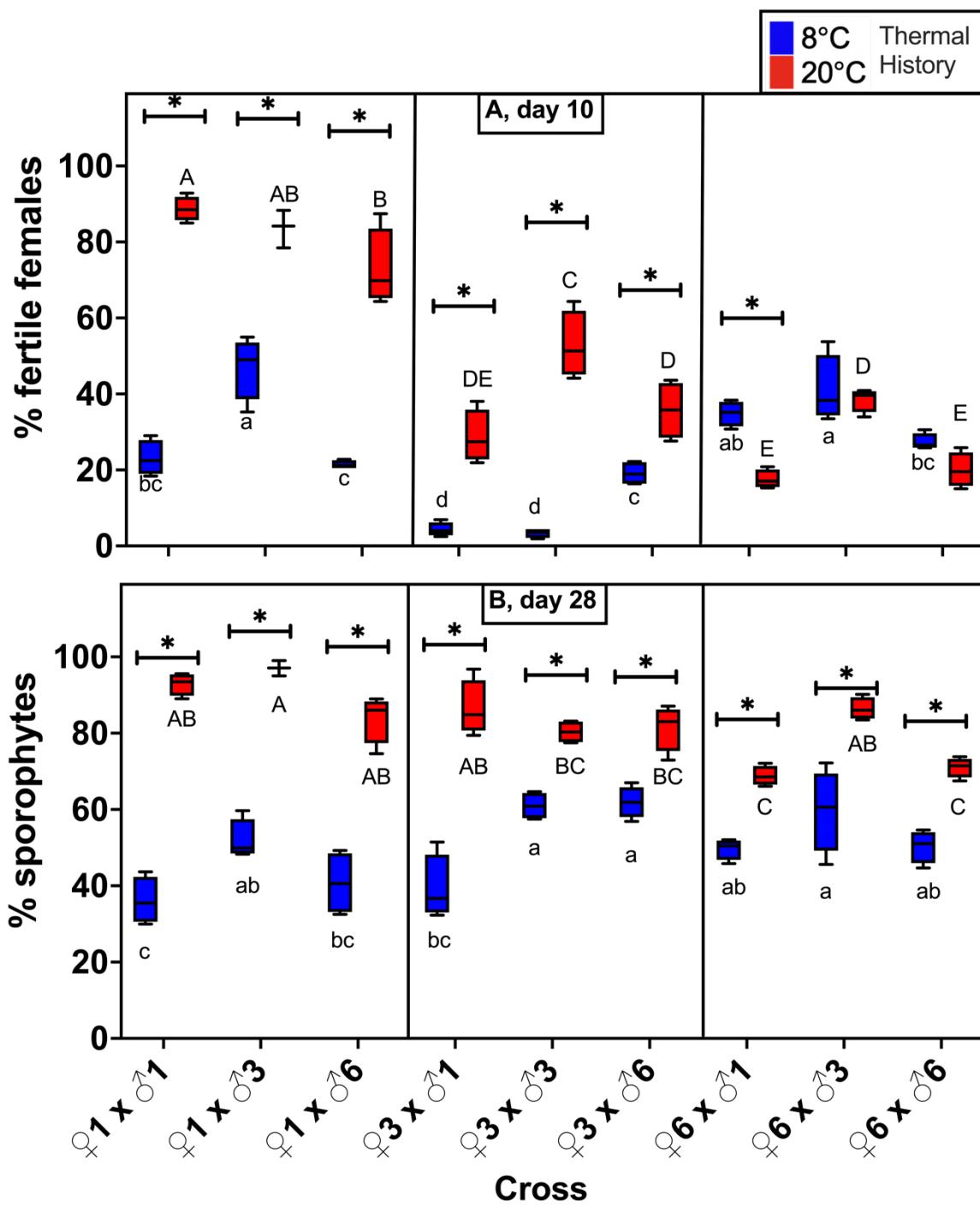
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1  
 2 **Figure 2: Effect of long-term temperature treatment (8°C and 20°C) on the maximum**  
 3 **photosynthetic yield of PSII ( $F_v/F_m$ ) of different *Laminaria pallida* gametophyte strains.**  
 4 Note that  $F_v/F_m$  values were normalized to the respective initial value for each strain. Bar plots  
 5 with mean and error bars with standard deviation ( $n = 4$ ). Different letters indicate significant  
 6 differences between strains. See Table 2 for statistics.  
 7



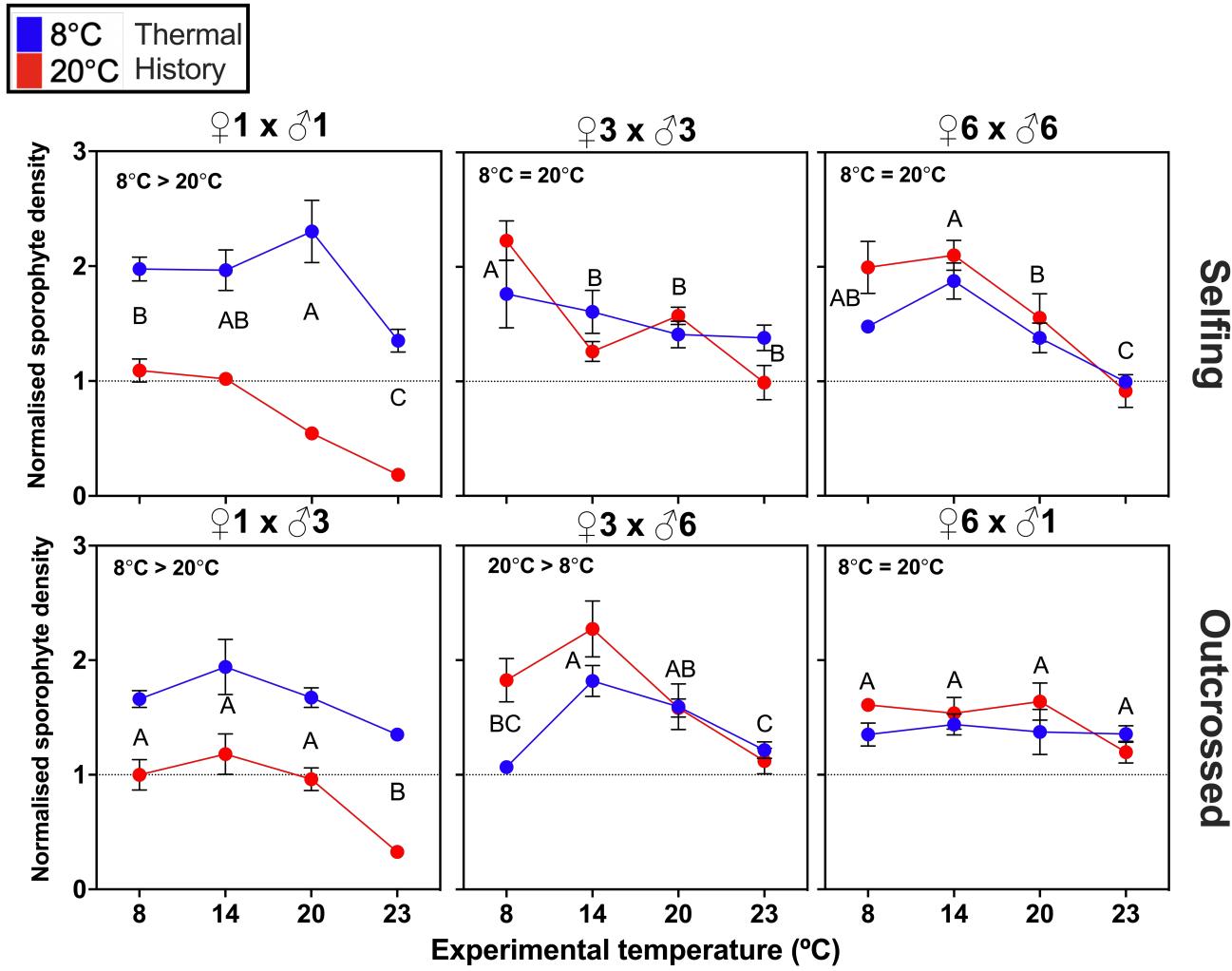
1  
2 **Figure 3: Effect of thermal history on the absolute growth rate of gametophytes from**  
3 **different *L. pallida* crosses after 6 days under gametogenic conditions.** Box plots with  
4 median, boxes for 25<sup>th</sup> and 75<sup>th</sup> percentiles and whiskers indicating min and max values (n =  
5 4). Panels separate crosses with different females. Different letters indicate significant  
6 differences between crosses ( $p < 0.05$ ). See Table 3 for statistics.  
7



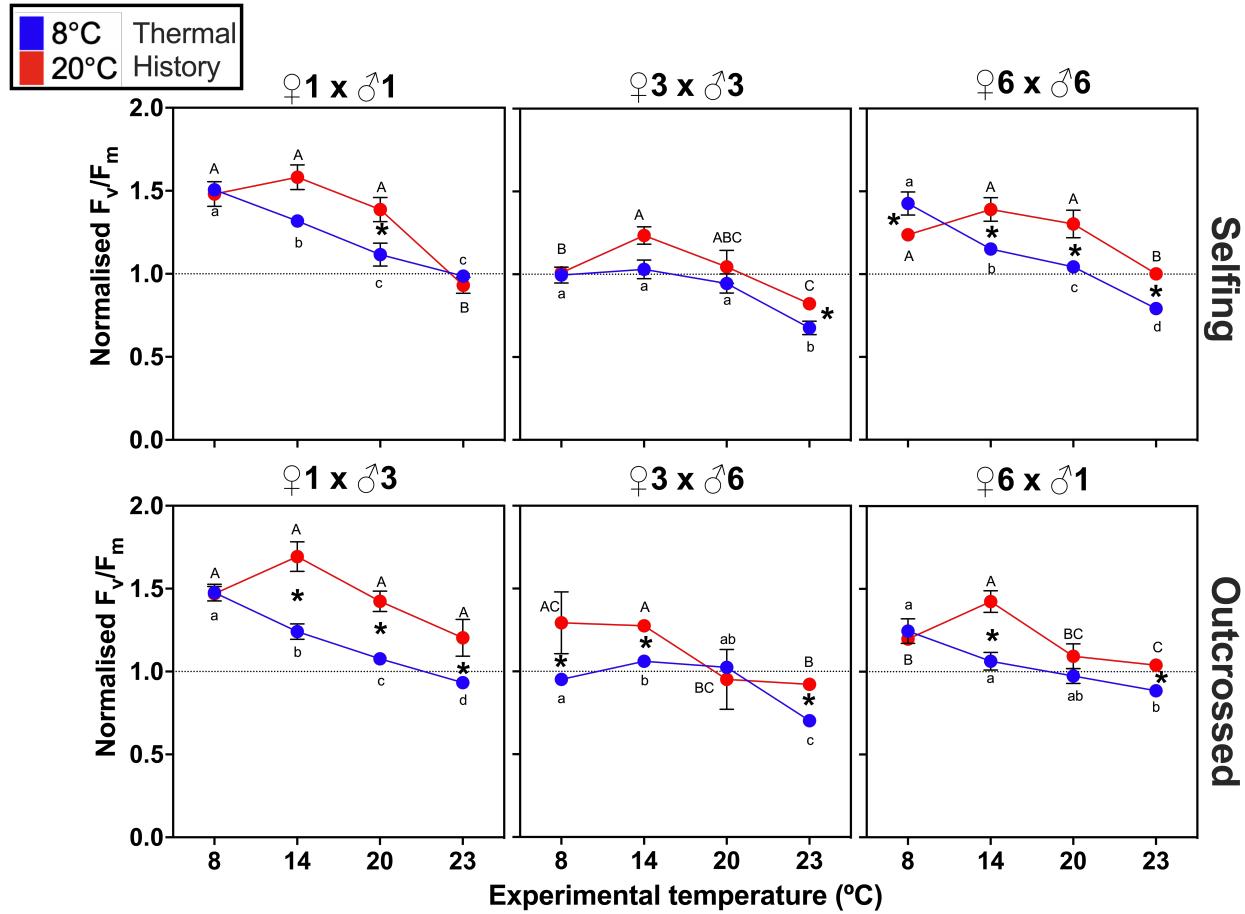
1  
2 **Figure 4: Effect of thermal history on the reproductive success of different *L. pallida***  
3 **crosses in gametogenic conditions.** (A) Percentage of fertile female gametophytes (egg  
4 released and sporophytes formed) after 10 days. (B) Percentage of female gametophytes with  
5 sporophyte(s) after 28 days. Box plots with median, boxes for 25<sup>th</sup> and 75<sup>th</sup> percentiles and  
6 whiskers indicating min and max values (n = 4). Panels separate crosses with different females.

1 \*Indicates a significant difference between thermal histories per cross. For each thermal  
2 history, different letters indicate significant differences between crosses ( $p < 0.05$ , uppercase  
3 letters for the 20°C thermal history and lowercase for the 8°C thermal history). See Table 4 for  
4 statistics.

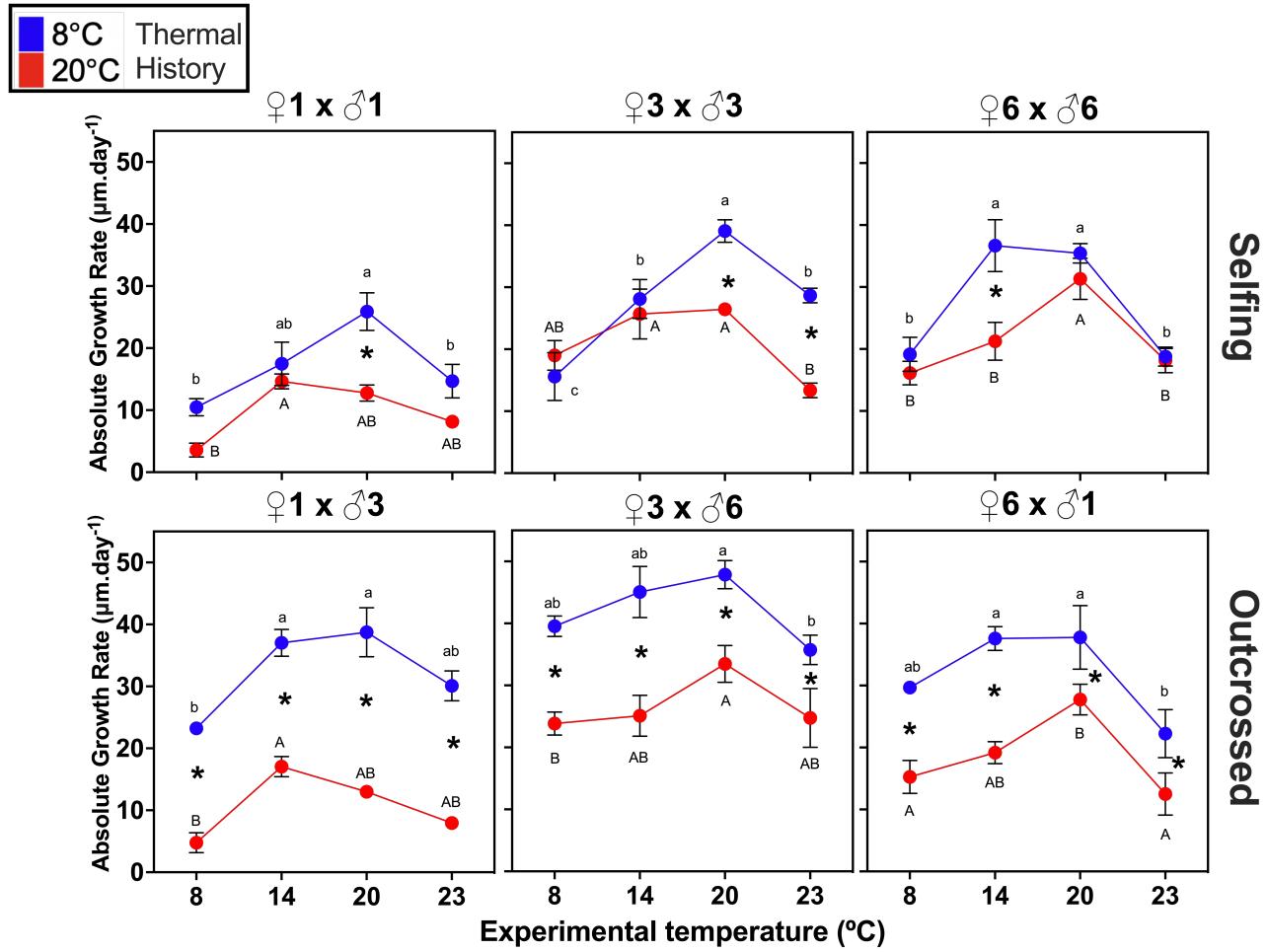
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1  
2 **Figure 5: Effect of parental thermal history on the sporophyte density of different *L.***  
3 ***pallida* crosses after 16 days in experimental temperatures (8°C, 14°C, 20°C and 23°C).**  
4 Connected mean plots with standard error of the mean (n = 4). Each plot corresponds to a cross  
5 of parental gametophytes. Values were normalized to the respective initial value for each strain  
6 and thermal history. For each cross, different letters indicate significant differences between  
7 experimental temperatures irrespective of thermal history. Differences between thermal  
8 histories irrespective of experimental temperatures are noted in the upper left corner of graphs  
9 (p < 0.05). See Table 5 for statistics.  
10



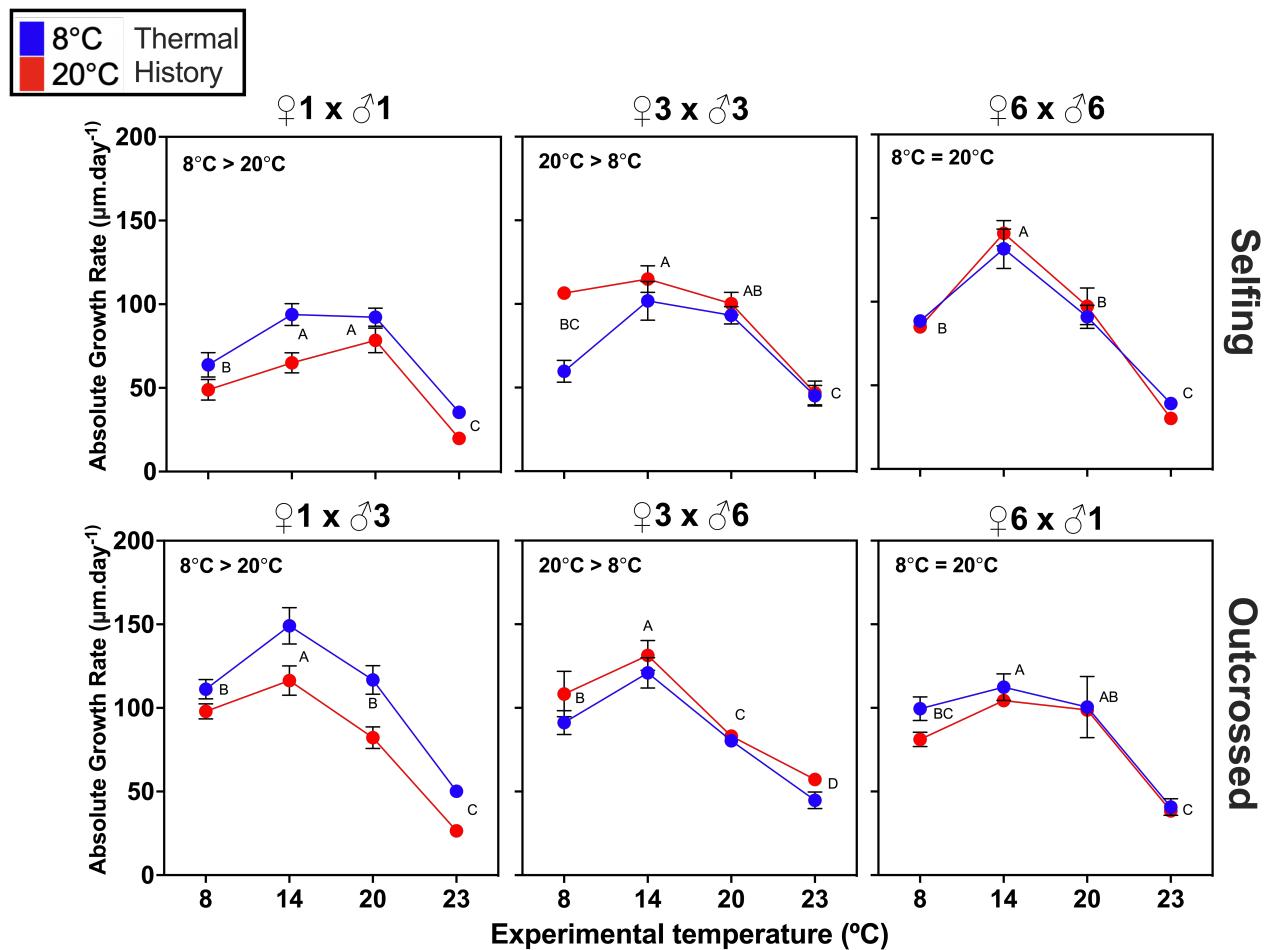
1  
 2 **Figure 6: Effect on parental thermal history on the sporophyte maximum photosynthetic  
 3 yield ( $F_v/F_m$ ) of different *L. pallida* crosses after 16 days in experimental temperatures  
 4 (8°C, 14°C, 20°C and 23°C). Connected mean plots with standard error of the mean (n = 4).**  
 5 Each plot corresponds to a cross of parental gametophytes. Values were normalized to the  
 6 respective initial value for each strain and thermal history. \* indicates a significant difference  
 7 between thermal histories per cross and experimental temperature. For each cross and each  
 8 thermal history, different letters indicate significant differences between experimental  
 9 temperatures (uppercase letters for the 20°C thermal history and lowercase for the 8°C thermal  
 10 history,  $p < 0.05$ ). See Table 6 for statistics.  
 11



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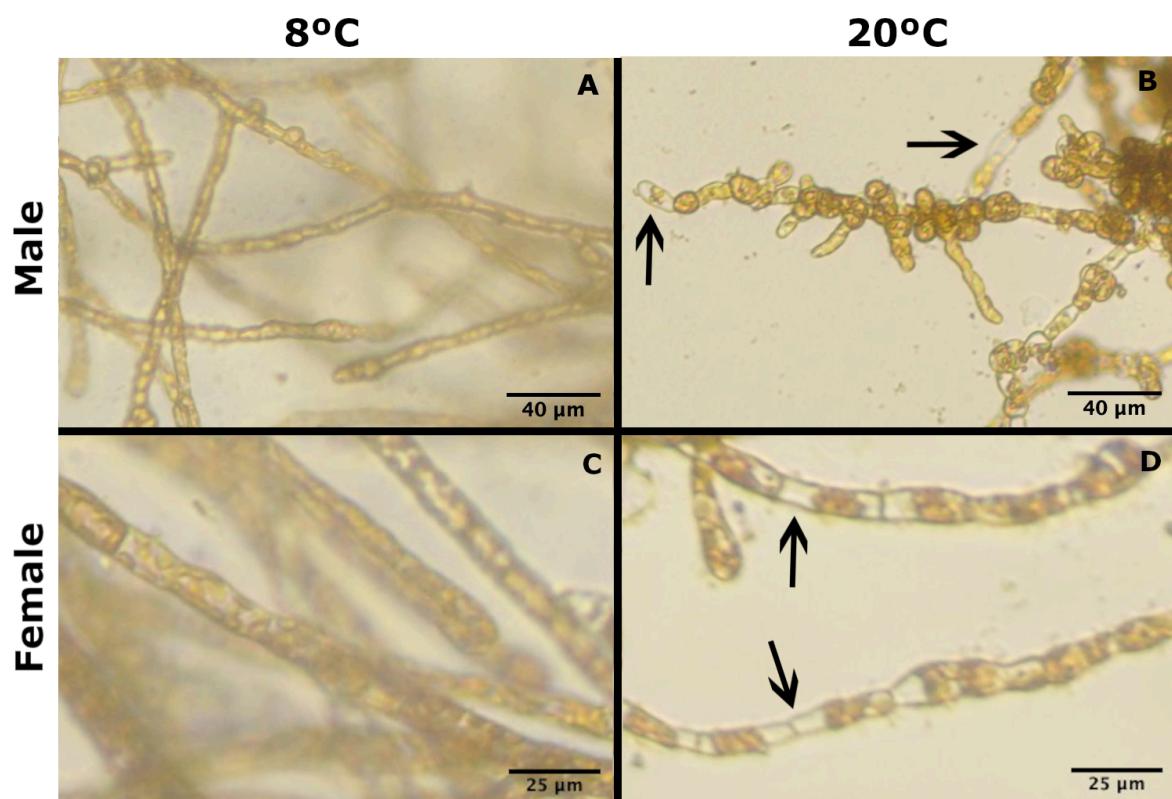
2 **Figure 7: Effect of parental thermal history on the sporophyte absolute growth rate based**  
 3 **on length of different *L. pallida* crosses after 8 days in experimental temperatures (8°C,**  
 4 **14°C, 20°C and 23°C).** Connected mean plots with standard error of the mean (n = 4). Each  
 5 plot corresponds to a cross of parental gametophytes. \* indicates a significant difference  
 6 between thermal histories per cross and experimental temperature. For each cross and each  
 7 thermal history, different letters indicate significant differences between experimental  
 8 temperatures (uppercase letters for the 20°C thermal history and lowercase for the 8°C thermal  
 9 history, p < 0.05). See Table 7 for statistics.

10

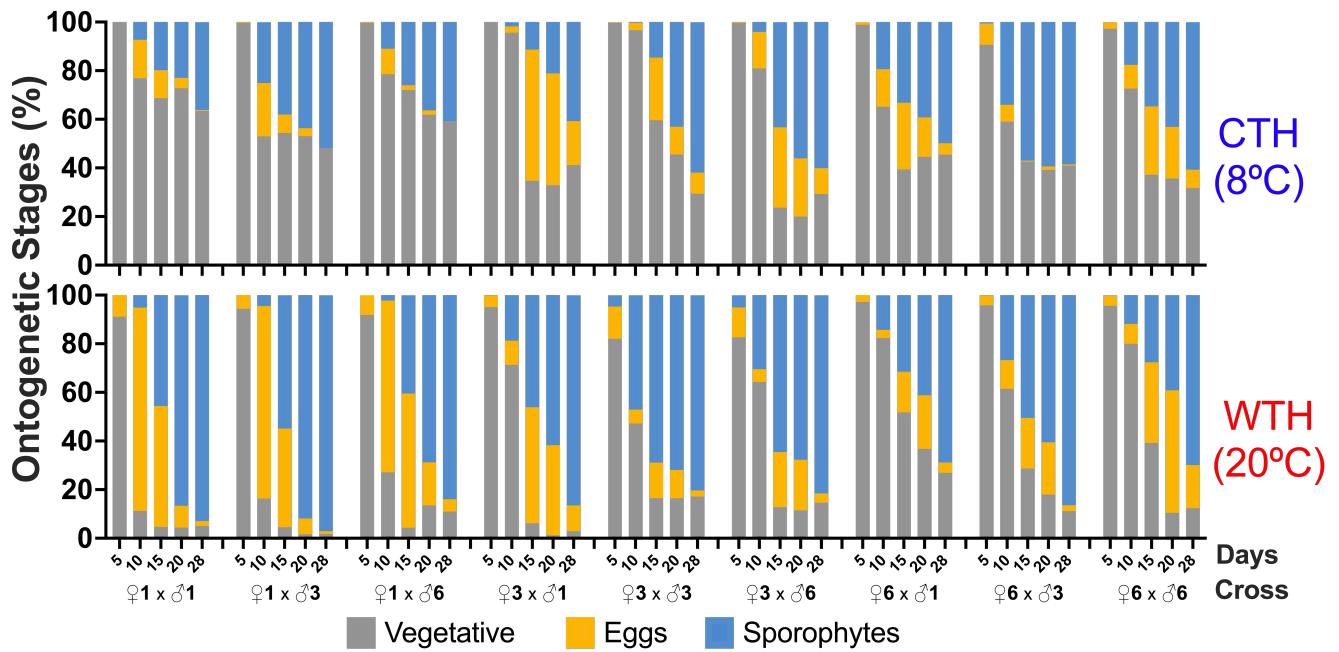


1  
2 **Figure 8: Effect of parental thermal history on the sporophyte absolute growth rate based**  
3 **on length of different *L. pallida* crosses between 8-16 days in experimental temperatures**  
4 **(8°C, 14°C, 20°C and 23°C).** Connected mean plots with standard error of the mean (n = 4).  
5 Each plot corresponds to a cross of parental gametophytes. For each cross, different letters  
6 indicate differences between experimental temperatures irrespective of thermal history.  
7 Differences between thermal histories irrespective of experimental temperatures are noted in  
8 the upper left corner of graphs (p < 0.05). See Table 7 for statistics.

9



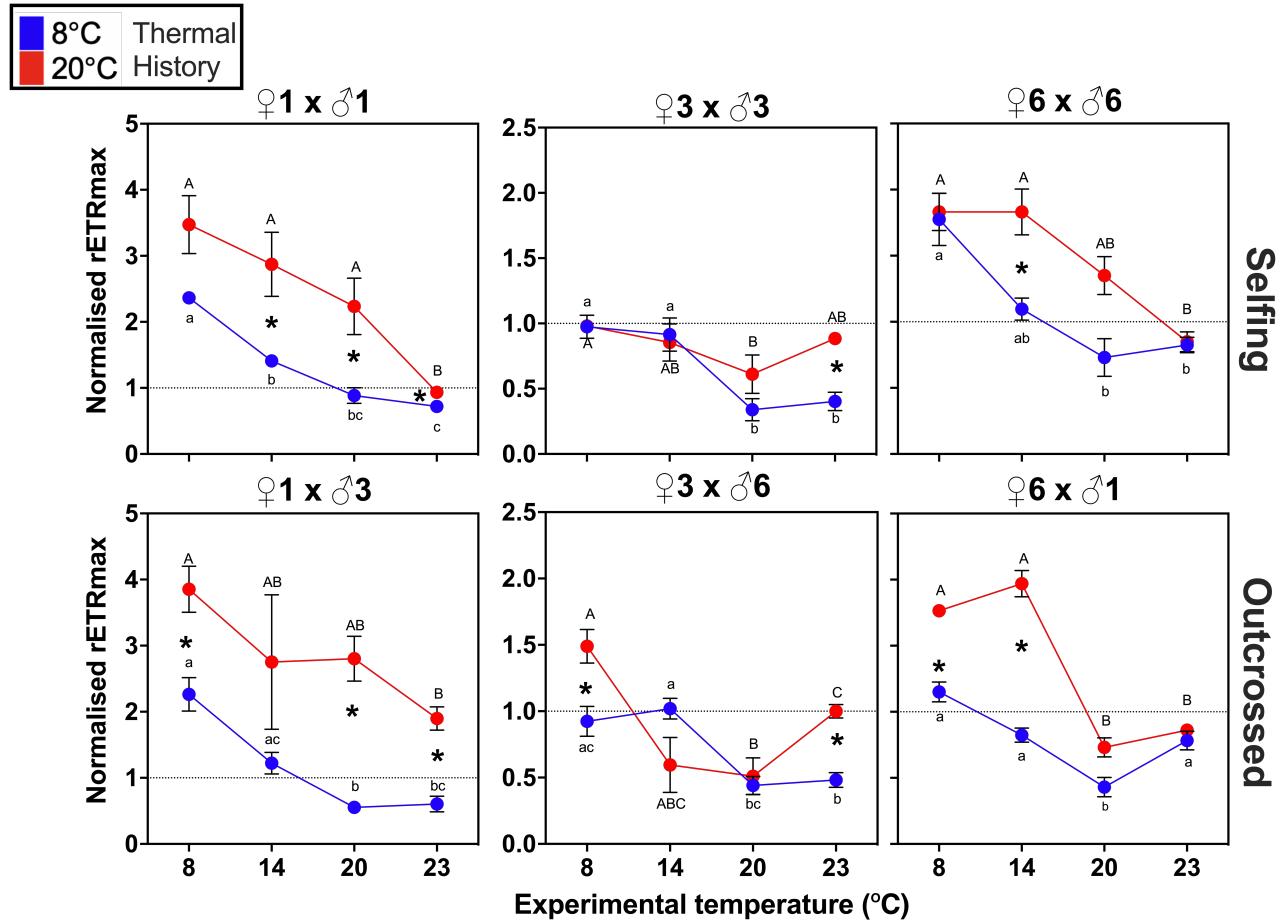
1  
2 **Figure S1: Gametophytes of *Laminaria pallida* after ~3.5 months of temperature exposure**  
3 **at 8°C and 20°C.** A, B. Male gametophyte cells. C, D. Female gametophyte cells. Arrows  
4 indicate dead or damaged cells.  
5



1

2 **Figure S2: Effect of thermal history on the female gametogenesis development of different**  
3 ***L. pallida* crosses over time (28 days).** 100% stacked column charts with means of each  
4 ontogenetic stage ( $n = 4$ ). Counting was performed every 5 days for the first 20 days and on  
5 day 28. SE-values are omitted for clarity. CTH = Cold Thermal History, WTH = Warm  
6 Thermal History.

7



1  
 2 **Figure S3: Effect of parental thermal history on the sporophyte relative maximum**  
 3 **electron transport rate (rETRmax) of different *L. pallida* crosses after 16 days in**  
 4 **experimental temperatures (8°C, 14°C, 20°C and 23°C).** Connected mean plots with  
 5 standard error of the mean ( $n = 4$ ). Each plot corresponds to a cross of parental gametophytes.  
 6 \* indicates a significant difference between thermal histories per cross and experimental  
 7 temperature. For each cross and each thermal history, different letters indicate significant  
 8 differences between experimental temperatures (uppercase letters for the 20°C thermal history  
 9 and lowercase for the 8°C thermal history,  $p < 0.05$ ). See Table 6 for statistics.