

1   **Effects of thermal history on reproduction and transgenerational plasticity in the kelp**

2   *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 within- and  
6    transgenerational plasticity in the warm-temperate kelp *Laminaria pallida*. Our results show  
7    that a warm temperature during the vegetative growth of gametophytes improved subsequent  
8    reproductive success under optimum compared to cold conditions. Transgenerational plasticity  
9    was also evident; thermal tolerance of microscopic F1 sporophytes was modulated by the  
10   thermal history of the parental gametophytes, but the effects were complex varying with the  
11   physiological trait and the exposure time. Genetic lines differed in their capacity for thermal  
12   plasticity (within- and transgenerational), with the direction and strength of responses more  
13   dependent on the female parental genome. This genetic variation suggests that thermal  
14   plasticity can potentially evolve under ocean warming. Our results indicated that the degree of  
15   gametophyte kinship (inbreeding versus outcrossing) does not influence the thermal plasticity  
16   response within and across generations. Overall, *L. pallida* benefits from warm conditions  
17   during the haploid (gametophyte) life stage, but milder temperatures (14-20°C) promote  
18   juvenile sporophyte performance. Our results suggest that within- 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.

1 KEY WORDS: brown alga; global warming; parental effects; phenotypic plasticity;

2 reproductive success; sporophyte formation

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 via phenotypic plasticity or genetic  
6    adaptation (Donelson et al., 2019). Sessile organisms are particularly vulnerable to climate  
7    change as natural distribution shifts and adaptation via natural selection may be too slow in the  
8    face of rapidly changing conditions (Atkins & Travis, 2010), posing a risk of range contraction  
9    and local extirpations. Consequently, phenotypic plasticity is critical in increasing the  
10   resilience of sessile species to changing environments (Schlichting, 2003). Phenotypic  
11   plasticity describes the capacity for phenotypic change in individuals responding to new  
12   environmental conditions without genetic changes. Plasticity allows species to survive  
13   emerging stressors long enough for distribution shifts to happen or genetic adaptations to  
14   emerge (Herman & Sultan, 2011; Weigel & Colot, 2012). Phenotypic plasticity can occur both  
15   within a generation (within-generational) and across generations (transgenerational). Thus,  
16   understanding the interactions and contributions of within- and transgenerational plasticity to  
17   the phenotypic response is important for the prediction of species adaptation to changing  
18   environments (Auge et al., 2017).

19   Within-generational plasticity occurs when an individual's phenotype shifts as a direct  
20   response to environmental conditions, while transgenerational plasticity (TGP, parental  
21   effects) the parental environmental history shapes offspring phenotype (Fox et al., 2019). TGP  
22   can be adaptive if parental effects act to increase offspring fitness. Examples of adaptive TGP  
23   are present in various taxa helping to overcome many different environmental stressors  
24   (Galloway & Etterson, 2007; Herman & Sultan, 2011). However, TGP can also be neutral or  
25   even maladaptive to the performance of species or populations in a changing world and has the

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

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

1 phenotypic plasticity has only been recently addressed in two kelp species (*Laminaria digitata*:  
2 Gauci, 2020; *Ecklonia radiata*: Mabin et al., 2019).

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

14 The split-fan kelp, *Laminaria pallida* (Greville), is mainly distributed on the South-West coast  
15 of Africa, between Danger Point in South Africa and Rocky Point in Namibia (Molloy, 1990),  
16 but it has been also observed on some islands in the Southern Ocean (e.g., Ile Saint-Paul,  
17 Papenfuss et al., 1942). In South Africa, it is one of two dominant kelp species together with  
18 *Ecklonia maxima*, while in Namibia it is the sole habitat-forming species (Rothman et al. 2017).  
19 *L. pallida* is reported as an economically and ecologically valuable kelp species in African  
20 coastal waters (Blamey & Bolton, 2018; Critchley et al., 1991). The distributional area of the  
21 species is characterized by strong upwelling and warm-temperate surface waters, from 11°C to  
22 22°C (Demarcq et al., 2003, meteonews.fr). In the northern distribution limit of the species,  
23 populations are generally more sparse and fragmented than southern ones and are usually  
24 exposed to warmer temperatures (Rothman et al., 2015). As seawater temperature is predicted  
25 to increase by 1-4°C by 2100 (Meredith et al., 2019), *L. pallida* populations near the northern

1 distribution limit in Namibia will be exposed to temperatures close or above the upper  
2 sporophyte survival temperature of 23°C (tom Dieck & de Oliveira, 1993), potentially leading  
3 to habitat loss.

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 thermal plasticity within  
6 and across early life stages of a Northern range population of *L. pallida*. Gametophytes were  
7 acclimated to different thermal conditions (cold winter temperature: 8°C and warm summer  
8 temperature: 20°C) within their natural seasonal range for ~3.5 months, to evaluate whether the  
9 thermal history of gametophytes leads to phenotypic plasticity in the speed and success of  
10 gametophyte reproduction (within-generational plasticity). Transgenerational effects were  
11 assessed by investigating whether the thermal tolerance of microscopic F1 sporophytes is  
12 affected by the thermal history of their gametophyte parents. We also examined whether  
13 thermal history effects within and across generations differ among genotypes and cross types  
14 (intergametophytic selfing vs outcrossing). Deeper knowledge of thermal history effects in *L.*  
15 *pallida* will help to improve the management of existing populations as well as secure  
16 sustainable aquaculture and population restoration initiatives in endangered locations.

17

## 18 **2. MATERIALS AND METHODS**

### 19 **2.1. Experimental design**

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

1 parental thermal history on their thermal tolerance. Several gametophyte strains from a single  
2 population were used to assess genetic variation in 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 (23°C) of *L. pallida* sporophytes (Martins et al., 2019). Sori were cleaned and meiospores from  
9 each sporophyte were released separately in sterile seawater. After spore germination, separate  
10 male and female gametophyte stock cultures were established for each individual (culture  
11 numbers: 1, 3 and 6) and maintained in a vegetative state in sterile half-strength Provasoli  
12 enriched seawater (PES; Provasoli, 1968) at 12°C under 3 µmol photons m<sup>-2</sup> s<sup>-1</sup> of red light and  
13 16h:8h light:dark (L:D) cycle in a climate-controlled chamber (Fitoclima S600, Aralab, Lisbon,  
14 Portugal). Sterile artificial seawater (Tropic Marin Sea Salt, Wartenberg, Germany) with a  
15 salinity of 34 ± 1 ppm was used for maintenance and all experiments. The culture medium was  
16 changed monthly, until the beginning of the experiment (ca. 1.5y).

17

18 **2.3. Gametophyte exposure to distinct thermal conditions (cold: 8°C and warm: 20°C)**

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

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

12

### 13 2.3.a. Photosynthetic efficiency

14 The maximum photosynthetic yield of PSII ( $F_v/F_m$ ) was measured at the beginning and end of  
15 the long-term thermal exposure (~3.5 months) using a FluorPen FP 110 (PSI, Drásov, Czech  
16 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  
17 proxy for physiological performance. Gametophytes were dark acclimated for five minutes  
18 before the measurements. Two measurements were taken for each replicated Petri dish, and the  
19 average  $F_v/F_m$  used. Data was normalised (divided by the mean values at day 0) to account for  
20 significant differences in the initial  $F_v/F_m$  values between strains, thereby allowing comparisons  
21 over time.

22

### 23 **2.4. Gametophyte reproduction at optimal temperature**

24 After ~3.5 months at 8°C and 20°C (thermal history), the gametophytes were transferred to  
25 14°C by slowly increasing or decreasing the temperature at a rate of 3°C day<sup>-1</sup>. The

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

22

#### 23 2.4.a. Gametophyte growth

24 To assess whether thermal history influenced gametophyte growth under optimal gametogenic  
25 conditions, gametophyte area was measured on day 0 and before egg release on day 6. The area

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

$$9 \quad AGR = \frac{(final\ area - initial\ area)}{experimental\ time\ (days)}$$

10  
11 2.4.b. Gametogenesis and reproductive success  
12 The relative occurrence of three ontogenetic stages in female gametophytes  
13 (vegetative/oogonia, eggs released, and sporophytes attached) was estimated every 5 days for  
14 the first 20 days and on day 28 of gametogenic conditions in a minimum of 200 female  
15 gametophytes per replicate using a Zeiss Observer D1 inverted microscope. The most advanced  
16 developmental stage was assigned for each female gametophyte. Sporophytes were considered  
17 present as soon as the first cell division was visible in the zygote. Gametogenesis rates were  
18 statistically compared between crosses and thermal histories by comparing the percentage of  
19 female reproductive gametophytes (egg released or sporophyte attached) on day 10, since at  
20 least one cross showed over 80% of reproductive females. Reproductive success was evaluated  
21 by considering the percentage of female gametophytes with sporophytes after 28 days and the  
22 absolute number of sporophytes per female gametophyte after 30 days. The total sporophyte  
23 density was evaluated by counting sporophytes in  $\geq$  60 fields of view (Zeiss Observer D1  
24 inverted microscope; 100 $\times$  magnification) per replicate. Female gametophyte density was  
25 estimated on day 28.

1

2 **2.5. Juvenile offspring sporophyte exposure to thermal treatments**

3 After 23 days in reproductive conditions, microscopic sporophytes with a mean length of ~225  
4  $\mu\text{m}$  developed in the different crosses and thermal histories. For each thermal history, offspring  
5 sporophytes from six crosses (3 inbred and 3 outcrossed) were randomly selected from the  
6 initial nine prepared, ensuring that each male and each female were represented in two crosses  
7 (Table 1, bold). Each of the four cover slips per Petri dish containing microscopic sporophytes  
8 was transferred to a different target experimental temperature (8, 14, 20 and  $23^\circ\text{C} \pm 0.5^\circ\text{C}$ ).  
9 Target temperatures were reached by increasing or decreasing the temperature at a rate of  $3^\circ\text{C}$   
10 day $^{-1}$ . Sporophytes were exposed to each target temperature for 16 days. Temperatures of 8, 14  
11 and  $20^\circ\text{C}$  were chosen to represent the thermal range experienced in nature across the  
12 distributional range (Dieckmann, 1978, 1980) and  $23^\circ\text{C}$  was chosen as the upper survival  
13 temperature (Martins et al., 2019; tom Dieck & de Oliveira, 1993) to assess differences between  
14 thermal histories. The experiment was performed with microscopic sporophytes as  
15 transgenerational effects are stronger early in life (Wilson and Reale 2006; Chang et al. 2021).  
16 Four large Petri dishes (8.9 cm diameter, height 2.5 cm) containing one cover slip each and 25  
17 ml of half-strength PES were used for each treatment (6 crosses  $\times$  2 thermal histories  $\times$  4  
18 experimental temperatures  $\times$  4 replicates = 192 Petri dishes). Experiments were conducted in  
19 temperature-controlled climatic chambers (Fitoclima S600, Aralab, Lisbon, Portugal), with 17  
20  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  of white light in a 16h:8h L:D cycle.

21

22 **2.5.a. Sporophyte density**

23 Sporophyte density was measured to assess the effect of experimental temperatures and thermal  
24 histories on the survival capacity of microscopic offspring sporophytes. Sporophyte densities  
25 were quantified at the beginning (day 0) and the end (day 16) of the thermal treatment. For

1 each replicate, sporophytes were counted in a minimum of 50 fields of view (Zeiss Observer  
2 D1 inverted microscope; 100 $\times$  magnification). Data was normalised with respect to initial  
3 sporophyte densities between crosses, thereby allowing comparisons between crosses.

4

5 2.5.b. Photosynthetic efficiency

6 Maximum photosynthetic efficiency was measured on day 0 and 16 to estimate the treatment  
7 effects on the physiological performance of microscopic sporophytes. A FluorPen FP 110 (PSI,  
8 Drásov, Czech Republic) was used to measure the maximum photosynthetic yield of PSII  
9 ( $F_v/F_m$ ) as well as the response to a light curve (Light curve 1, Flash pulse: 20%, Super pulse:  
10 40%, Actinic pulse: 18  $\mu\text{mol}$ ) in each replicate. The light curve response was used to calculate  
11 the relative maximum electron transport rate (rETRmax) using the Phytotools package in R  
12 software (R Core Team, 2021; Silsbe & Malkin, 2015). Sporophytes were dark acclimated for  
13 five minutes before the measurements. Data was normalised (divided by the mean values at  
14 day 0) to account for significant differences initially between sporophytes from different  
15 crosses, allowing comparisons between crosses.

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

4 **2.6. Statistical Analysis**

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

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

24

25 **3. RESULTS**

1    **3.1. Gametophyte fitness after long term thermal exposure**

2    The normalised maximum quantum yield of PSII ( $F_v/F_m$ ) differed significantly with  
3    temperature and strain, but there were no interactions between the two factors (Table 2, Fig.  
4    2). Normalised  $F_v/F_m$  was significantly lower (ca. 1.4-fold) in gametophytes exposed to warm  
5    temperature (20°C) compared to cold temperature (8°C), irrespective of the strain. These results  
6    were corroborated by the visual appearance of the gametophytes that presented healthy brown  
7    pigmented cells at 8°C, while the gametophytes growing at 20°C had some plasmolyzed cells,  
8    indicating high stress and cell wall damage (Fig. S1). The gametophyte strains ♀1, ♀3, ♂3 and  
9    ♂6 showed lower (1.7-fold)  $F_v/F_m$  values compared to strains ♂1 and ♀6.

10

11    **3.2. Gametophyte reproduction at optimal temperature**

12    **3.2.a. Gametophyte growth**

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

17

18    **3.2.b. Female reproductive success and sporophyte formation**

19    Gametogenesis was in general faster and more successful in female gametophytes with warm  
20    thermal history (WTH; 20°C) compared with cold thermal history (CTH; 8°C) (Fig. S2). At  
21    both temperatures, patterns of temporal reproductive development was similar for crosses  
22    involving the same female gametophyte strain but varied in crosses involving the same male  
23    strain. To compare initial rates of gametogenesis, the percentage of reproductive females (i.e.,  
24    gametophytes with released eggs and/or attached sporophytes) was analysed after 10 days.  
25    Significant cross × thermal history interactions were detected (Table 4a, Fig. 4A). WTH

1 gametophytes exhibited higher female reproductive success in all the crosses with ♀1 and ♀3  
2 compared to CTH gametophytes. In contrast, CTH enhanced female reproductive success (2.0-  
3 fold) only in the cross ♀6 × ♂1 compared to WTH. In the CTH gametophytes, reproductive  
4 success was similar in the crosses with ♀1 and ♀6, while higher values were observed when  
5 both these females were crossed with ♂3 than ♂1 and ♂6. The lowest percentage of female  
6 reproduction occurred in the crosses ♀3 × ♂1 and ♀3 × ♂3. In WTH gametophytes, female  
7 reproductive success was significantly higher in crosses with ♀1 compared with all other  
8 crosses.

9

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

9 Sporophyte density also showed a significant interaction between both factors (cross × thermal  
10 history, Table 4c, Fig. 4C). WTH gametophytes produced significantly more sporophytes per  
11 female in all the three crosses involving ♀1 and in two of the crosses with ♀3 compared with  
12 CTH gametophytes, while gametophyte thermal history had no influence on sporophyte  
13 formation in all the remaining crosses (all with ♀6 and ♀3 × ♂3). In CTH gametophytes, the  
14 numbers of sporophytes per female gametophyte were significantly higher (1.8-fold) in two of  
15 the crosses involving ♀3 (♀3 × ♂3 and ♀3 × ♂6) than in all the crosses with ♂1 and in the ♀1  
16 × ♂6 cross. Sporophyte formation was higher (1.8-fold) in the cross ♀3 × ♂6 than in all the  
17 other crosses in the WTH gametophytes.

18 Taken together, these female reproductive results revealed three major findings: (1) WTH  
19 history gametophytes showed greater rates of gametogenesis, with higher fertility and  
20 reproductive success (sporophyte production)(within-generational plasticity); (2) Strain-  
21 specific variation in within-generational plasticity was observed as gametophyte strains ♀1 and  
22 ♀3 were in general more prone to the effect of thermal history than ♀6; 3) Male gametophytes  
23 affect female gametophyte reproduction, as each female strain showed different reproductive  
24 success when crossed with distinct male strains.

25

1    **3.3. Effects of parental thermal history on juvenile sporophytes**

2    3.3.a. Sporophyte density

3    Sporophyte densities increased in the ♀1 crosses with CTH over the 16 days (normalised  
4    density > 1) independent of the experimental temperature, indicating ongoing gametophyte  
5    reproduction and sporophyte formation (Fig. 5). Similarly, increases in sporophyte density  
6    were also observed in the remaining crosses at 8, 14 and 20°C, regardless of the parental  
7    thermal history. At the higher temperature (23°C), further maturation seems to be prevented,  
8    and the stable sporophyte densities reflect survival rather than additional development in these  
9    crosses. In the two WTH ♀1 crosses, sporophyte densities remained stable (normalised density  
10   ≈ 1) at the lowest temperatures (8 and 14°C), but decreased at higher temperatures, particularly  
11   at 23°C, indicating sporophyte mortality.

12   The normalised sporophyte density showed no significant cross × temperature × thermal  
13   history interactions, but all three two-factor interactions were significant (Table 5). Parental  
14   thermal history had no influence on the sporophyte density of both crosses with ♀6 and in the  
15   selfing ♀3 × ♂3, while higher sporophyte densities (2.3-fold) were observed in the two ♀1  
16   crosses with a CTH (Fig 5). In contrast, a WTH enhanced sporophyte density (1.2-fold) in the  
17   outcrossed ♀3 × ♂6 compared to a CTH. Overall, sporophyte densities were lower at 23°C  
18   (1.6-fold) than at all the other temperatures, while the highest densities in general were found  
19   at 14°C, irrespective of the thermal history.

20

21   3.3.b. Sporophyte photosynthetic responses

22   A significant cross × temperature × thermal history interaction was detected for both maximum  
23   photosynthetic yield ( $F_v/F_m$ ) and relative maximum electron transport rate (rETRmax, Table  
24   6). Overall, normalised  $F_v/F_m$  and rETRmax values were significantly higher (1.2-fold and 1.7-  
25   fold, respectively) in WTH sporophytes. This was especially prevalent for the sporophytes

1 exposed to the highest temperature of 23°C (5 out of 6 crosses; Table S1, Figs. 6 and S3). Only  
2 in the inbred cross ♀6 × ♂6 at 8°C did CTH result in higher sporophyte  $F_v/F_m$  compared to  
3 WTH (1.2-fold).

4 In general,  $F_v/F_m$  was highest at 8°C for the CTH, but at 14°C for the WTH sporophytes (Fig.  
5 6).  $F_v/F_m$  was negatively affected by increasing experimental temperatures, being more evident  
6 in the CTH sporophytes. At the highest temperature of 23°C,  $F_v/F_m$  significantly decreased  
7 compared to all the other temperatures in the sporophytes from both thermal histories (1.4-fold  
8 overall). Overall, the highest rETRmax values occurred at 8°C, regardless of the sporophyte  
9 parental thermal history (Fig. S3). For both thermal histories, rETRmax showed a decreasing  
10 trend at  $\geq 20^\circ\text{C}$  compared with the lower temperatures.

11

### 12 3.3.c. Sporophyte growth

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

21 From day 8 to day 16, the AGR of sporophytes differed due to cross × temperature and cross  
22 × thermal history interactions (Table 7b). Thermal history effects were linked to maternal  
23 gametophytes; ♀1 crosses: higher AGR (1.3-fold) were observed in the CTH sporophytes, ♀3  
24 crosses: WTH sporophytes showed higher growth rates (1.2-fold) compared to CTH  
25 sporophytes, ♀6 crosses: no significant differences on the sporophyte AGR were detected

1 between parental thermal histories (Fig. 8, Table S2). Overall, sporophyte growth rates were  
2 highest at 14°C, significantly decreasing at 8°C (1.3-fold; in some crosses also at 20°C) and  
3 even more at the highest temperature (23°C, 2.9-fold).

4

5 **4. DISCUSSION**

6 Since environmental conditions are changing rapidly in marine environments, phenotypic  
7 plasticity may help to mitigate population decline by alleviating a “phenotype-environment  
8 mismatch,” thus buying time for evolutionary rescue. Our study revealed within- and  
9 transgenerational plasticity for thermal tolerance in the year-round reproductive kelp species,  
10 *L. pallida*. Long-term exposure to warm seawater temperature (20°C) reduced the physiological  
11 performance of gametophytes compared to cold conditions (8°C), but it promoted subsequent  
12 gametogenesis and reproductive success under optimal conditions. This may be a product of  
13 natural seasonality in reproduction and/or of stress-induced gametogenesis. The performance  
14 of offspring sporophytes under a temperature gradient was also influenced by the thermal  
15 environment experienced by the parental gametophytes, however the effects are complex,  
16 varying according to the physiological trait investigated and the exposure time. Furthermore,  
17 genetic variation in within- and transgenerational plasticity was also demonstrated, where the  
18 response direction and strength appear largely dependent on the female parental genome.  
19 Together, our data show that understanding the environmental history effects on kelps requires  
20 taking both environmental and genetic context into account, as a range of responses to thermal  
21 stress are expected between individuals with differing thermal histories.

22

23 **4.1. Decreased gametophyte fitness under warm seawater temperature**

24 Although *L. pallida* sporophytes are reproductive year-round, the peak is mainly at the end of  
25 the austral summer (March, Dieckmann, 1980), when surface temperatures are still warm (17-

1 21°C in Swakopmund, seatemperature.org). Spores that settle on rocky substrate and develop  
2 gametophytes will most probably remain vegetative for some months until average  
3 temperatures allow gametogenesis ( $\leq 17^{\circ}\text{C}$  – May; tom Dieck and de Oliveira, 1993).  
4 Vegetative gametophytes in nature are therefore exposed to similar temperatures as the WTH  
5 ( $20^{\circ}\text{C}$ ) used in this study. The gametophytes of *L. pallida* maintained under WTH for ~3.5  
6 months showed reduced physiological fitness (low photosynthetic efficiency and presence of  
7 plasmolyzed cells indicating stress) compared to gametophytes growing under CTH ( $8^{\circ}\text{C}$ ).  
8 Although  $20^{\circ}\text{C}$  is within the thermal range of *L. pallida*, the upper survival temperature of  
9 gametophytes has been shown to decrease with exposure time, surviving  $27^{\circ}\text{C}$  for 1 day,  $25^{\circ}\text{C}$   
10 for 2 weeks, while this limit decreases to  $23^{\circ}\text{C}$  after 8 weeks (tom Dieck, 1993). Our results  
11 suggest that the gametophytes exposed to  $\sim 20^{\circ}\text{C}$  *in situ* may already experience sublethal  
12 conditions affecting physiological health. Predicted increases in water temperatures due to  
13 climate change may therefore subject gametophytes to sublethal conditions with potential  
14 impacts on reproductive output and/or success.

15 In rarer cases of spore release during colder months, gametophytes may become reproductive  
16 ( $8^{\circ}\text{C}$ ; Martins et al. 2019) or settle in a vegetative state below the canopy under dark conditions  
17 until irradiance is sufficient to trigger gametogenesis. Our results suggest that gametophytes  
18 would remain healthy during prolonged exposure to low temperatures.

19

20 **4.2. Within-generational plasticity: warm thermal history promotes gametogenesis and**  
21 **female reproductive success**

22 We found that gametophyte thermal history impacts female gametophyte reproduction,  
23 revealing within-generational plasticity in *L. pallida*. Although, the development under warm  
24 seawater temperature ( $20^{\circ}\text{C}$ ) reduced the gametophyte physiological performance, it promoted  
25 the speed of gametogenesis and the reproductive success under optimal conditions. Similarly,

1 in the kelp *Alaria esculenta* faster gametogenesis in WTH (22°C) gametophytes was suggested  
2 to explain enhanced growth in sporophytes with a parental WTH compared to CTH (12°C)  
3 (Quigley, 2018). However, these results contrast with previous studies on cold-adapted kelp  
4 species, where WTH led to decreases in the fertility of gametophytes (*L. digitata*: Martins et  
5 al., 2020; Gauci, 2020). Together, these results suggest that in kelps thermal stress memory  
6 might affect the timing of gametogenesis and reproductive success, but that responses to cold  
7 or heat stress memory may be species-specific.

8 The WTH promotion of *L. pallida* gametogenesis and sporophyte formation is consistent with  
9 its seasonal reproductive pattern in the native environment. Spores release at the end of the  
10 summer (Rothman et al., 2015) suggests that gametophyte development occurs seasonally  
11 under high summer/autumn seawater temperatures, while sporophyte formation occurs during  
12 lower winter temperatures (Dieckmann, 1978). Reproduction in Laminariales is known to be  
13 associated with seasonal environmental cues, including changing seawater temperature,  
14 daylength or irradiance (Bettignies et al. 2018; Martins et al., 2017; Kain 1989). The warm  
15 thermal environment to which gametophytes were exposed may have acted as a seasonal  
16 thermal cue, preconditioning gametophytes for reproduction when cooler conditions followed.  
17 In other brown algae, like fucoids, gametogenesis initiates in response to short days (Bäck et  
18 al., 1991; Berger et al., 2001), whereas water movements and lunar or tidal cycles are key  
19 proximal signals in gamete release (Monteiro et al. 2016; Pearson et al., 1998; Pearson &  
20 Serrão, 2006; Serrao et al., 1996). In some Arctic kelp species, gametogenesis occurs only  
21 under short days (<8 h light) and low seawater temperatures (Wiencke & tom Dieck, 1989).  
22 The warm-temperate kelp *Ecklonia radiata* produces the healthiest gametophytes when days  
23 are long to promote germination and growth, but gametogenesis and sporophyte production  
24 occurs later when daylengths are shorter and water temperature declines (Mohring et al., 2013).  
25 In the transition from vegetative growth to gametogenesis, the thermal history of gametophytes

1 may affect the rate or extent to which ribosome, transcription and translation related pathways  
2 are triggered, impacting gametogenesis and reproductive success (Pearson et al., 2019).  
3 There is extensive evidence of heat-stress induced flowering in plants (reviewed by Takeno,  
4 2016) and seagrasses (Blok et al., 2018; Marín-Guirao et al., 2019; Ruiz et al., 2018), is and  
5 interpreted as an ultimate stress adaptation, as plants can survive as a species if they flower and  
6 produce seeds even when they cannot survive under severe stress events. Similarly, heat stress  
7 enhanced the production of new gametophytic thalli accelerating the asexual reproduction in  
8 the red alga *Pyropia yezoensis* (Suda & Mikami, 2020). The warm seawater temperature  
9 experienced by the gametophytes of *L. pallida* might have promoted the subsequent  
10 gametogenesis via a similar species survival mechanism. The increased production of  
11 sporophytes might compensate their physiological state under warming summer conditions.  
12 On the other hand, *L. pallida* gametophyte growth at optimal gametogenic conditions (14°C,  
13 white light) was not influenced by the thermal histories (8°C and 20°C). As gametogenesis and  
14 growth are mutually exclusive processes in kelps (Izquierdo et al. 2002; Martins et al. 2017),  
15 the potential resources linked to thermal history were probably allocated to the formation of  
16 reproductive structures (antheridia and oogonia) rather than for vegetative growth.

17

#### 18 **4.3 Female gametophyte reproduction is shaped by the male strain**

19 It is known for long that kelp fertilization is facilitated by the universal pheromone lamoxirene  
20 secreted by the female gametophyte when eggs are produced. Lamoxirene triggers  
21 spermatozoid release from antheridia and their attraction to the eggs (Lüning and Muller 1978).  
22 Although, no pheromone production or chemical signalling by male gametophytes has been  
23 demonstrated in kelps, recent evidence shows the influence of male gametophytes on female  
24 reproduction. Martins et al. (2019) discovered that female gametogenesis in *L. digitata* and *L.*  
25 *pallida* was promoted when male gametophytes were present. Furthermore, female fecundity

1 and fertility was also shown to be dependent on male presence, identity and kinship in the giant  
2 kelp, *Macrocystis pyrifera* (Camus et al. 2021). Similarly, in our study the reproductive rate  
3 and success of the female gametophytes was shaped by the male gametophyte strain. However,  
4 no clear pattern was found, i.e., the same male did not lead to the same reproductive output  
5 independent of the female strain. Taken together, these findings support the existence of a  
6 complex bidirectional mechanism of chemical communication between female and male kelp  
7 gametophytes that deserves further attention.

8

#### 9 **4.4. Transgenerational effects on the thermal tolerance of microscopic sporophytes**

10 *L. pallida* gametophytes from northern populations most probably develop and grow *in situ*  
11 under warm summer temperatures, whereas sporophyte recruitment happens in early winter  
12 (Dieckmann, 1978) and juvenile sporophytes grow under mild temperatures (12-16°C;  
13 Dieckmann, 1978). In our study, *L. pallida* microscopic sporophytes displayed  
14 transgenerational thermal plasticity. Sporophyte from WTH parental conditions showed higher  
15 photosynthetic efficiency, particularly at moderate to high temperatures (14-23°C) compared  
16 to CTH. Several studies have demonstrated that prior exposure to high temperatures can  
17 enhance tolerance in marine macrophytes when exposed to thermal stress (heat-stress memory;  
18 *Zostera muelleri* and *Posidonia australis*: Nguyen et al., 2020; *Bangia fuscopurpurea*:  
19 Kishimoto et al., 2019; *Fucus vesiculosus*: Li and Brawley, 2004). Parental thermal history  
20 shaped the thermal performance curve of photosynthetic efficiency in *L. pallida* offspring. The  
21 temperature at which the sporophytes show the highest *Fv/Fm* values shifted from 8°C in CTH  
22 sporophytes to 14°C in sporophytes with a parental WTH. This is in line with earlier studies in  
23 various organisms showing that prior environmental history can influence thermal  
24 performance, with higher acclimation temperatures resulting in upwards thermal optima shifts  
25 (Samuels et al. 2021; Klepsat et al. 2020; Seebacher et al., 2015; Sendall et al. 2015). Thus,

1 our study confirms that the temperature at which maximum performance occurs can respond  
2 to changes in environmental conditions.

3 Although studies on thermal plasticity across generations have recently increased for marine  
4 organisms (McRae et al., 2021; Shama et al., 2016), the temporal effects on offspring  
5 performance are still unclear. In our study, sporophyte growth responses varied over exposure  
6 time to a range of temperatures; sporophytes from CTH grew significantly more than WTH  
7 during the first 8 days, particularly at mild to warm temperatures (14-23°C). This may represent  
8 a potential “silver-spoon” parental effect (Baker et al., 2019; Germain et al., 2019), which  
9 describes a physiological advantage for individuals whose parents had access to abundant  
10 resources. Similarly, in the cold-temperate kelp *L. digitata* the growth of early sporophytes at  
11 extreme temperatures (0°C and 20°C) was improved by a cold parental thermal history (5°C;  
12 Gauci, 2020). Interestingly, the “silver spoon” parental effect disappeared over time (from day  
13 8 to day 16) and thermal plasticity dependent on the maternal line become evident. In the  
14 offspring from maternal line ♀1 growth was enhanced by a parental CTH, while the WTH  
15 sporophytes from maternal line ♀3 grew more than those from CTH. On the other hand,  
16 parental thermal history had no influence on the growth of the offspring from maternal line ♀6.  
17 These results suggest a more complex picture of the effects of thermal history on the offspring  
18 performance of this warm temperate kelp species that are dependent of the temperature  
19 exposure duration in the offspring.

20 Parental WTH was expected to enhance the growth of the offspring sporophytes as was  
21 observed for the photosynthetic efficiency, however sporophyte growth did not conform to our  
22 predictions. This might be associated with a possible fecundity-growth trade-off, where WTH  
23 sporophytes might have less energy available to invest in growth due to the successful  
24 reproduction during the parental stage and thus increases photosynthesis efficiency, while CTH  
25 sporophytes might still have parental resources available for growth not requiring increasing

1 the photosynthetic performance. Measurement of other stress-related traits (e.g., antioxidant  
2 enzymes) at sublethal temperatures may help elucidate the observed responses. Together, the  
3 results show that the thermal environment experienced by the parental gametophytes influences  
4 the response plasticity of offspring sporophytes, however the effects are complex, varying  
5 according to the physiological traits investigated and the exposure time, thus further studies are  
6 needed to provide more insight into this understudied topic.

7

8 **4.5. Genetic variation in within- and transgenerational plasticity: via female or maternal  
9 effects**

10 Genetic variation in within- and transgenerational plasticity for thermal tolerance was  
11 demonstrated for *L. pallida*. Within a generation, the phenotypic plasticity response of  
12 gametophytes to thermal history was dependent on the female genetics. Crosses with ♀1 and  
13 ♀3 showed within-generational plasticity for reproductive success, with a WTH enhancing  
14 fertility and offspring output. However, thermal history had less influence in crosses with ♀6,  
15 particularly on the production of sporophytes. Similarly, the effect intensity differed between  
16 crosses with ♀1 and ♀3 (stronger in ♀1 crosses with respect to reproductive success between  
17 WTH and CTH gametophytes). These variations in reproductive plasticity within a generation  
18 may be linked to the aptitude to form and retain epigenetic markers, e.g., DNA methylation or  
19 histone modification. Genetic variation in plasticity has been extensively described in plants  
20 (Sultan et al., 2009; Vu et al., 2015) and was recently shown in kelp species (Alsuwaiyan et  
21 al., 2021; Mabin et al., 2019; Liesner et al., 2020). Alsuwaiyan et al. (2021) found that haploid  
22 and diploid life stages of *Ecklonia radiata* genotypes differed in their susceptibility to thermal  
23 stress. Similarly, in *Laminaria digitata* variation in thermal plasticity was reported among  
24 genetic lines within a North Sea population (Liesner et al., 2020).

1 Similarly, the direction and strength of the transgenerational plasticity of offspring sporophytes  
2 was affected by the maternal genome or epigenome. Sporophyte mortality was only observed  
3 in ♀1 crosses with a parental WTH when exposed to the highest temperature (23°C). Moreover,  
4 different transgenerational effects on sporophyte growth rates (from day 8 to day 16 of thermal  
5 exposure) were observed for each maternal line, suggesting a strong maternal effect on the  
6 aptitude for transgenerational plasticity. These results suggest that epigenetic mechanisms are  
7 retained more during maternal than paternal gametogenesis. Maternal effects on  
8 transgenerational plasticity have been observed in a variety of taxa (e.g., Galloway & Etterson,  
9 2007; Marshall, 2008; Shama et al., 2014) and are considered the norm, as females have more  
10 ways to impact offspring development (e.g., egg provisioning, transferable maternal material  
11 such as mRNA and heritable heat-shock proteins). Evidence of maternal effects on offspring  
12 performance also exist in Laminariales (Mabin et al., 2019; Martins et al., 2019; tom Dieck &  
13 de Oliveira, 1993; Zhang et al., 2011). For example, in *E. radiata* it was proposed that maternal  
14 effects were associated with the gametophyte morphological variation to different temperature  
15 and light levels (Mabin et al. 2019). Moreover, thermal responses of interspecific hybrids  
16 between *L. digitata* and *L. pallida* revealed that female parents are more important in  
17 determining the offspring phenotype than male parents (Martins et al., 2019). Similarly, the  
18 female parent was responsible for the sensitivity to low temperature in hybrids between *L.*  
19 *pallida* and *L. abyssalis* (tom Dieck & de Oliveira, 1993).  
20 The genetic variation of transgenerational plasticity is double-edged as the genetic component  
21 of plasticity offers a target for selection, potentially increasing the resilience and fitness of  
22 genetically diverse populations (Chevin et al., 2010; Munday et al., 2017, 2019), but such  
23 selection is likely to lead to a loss of genetic variation. In isolated, sparse populations like that  
24 studied here, this might lead to problematic inbreeding rates.

1 Our results highlight the importance of considering the degree of genetic variation that is  
2 involved in kelp within- and transgenerational plasticity. Studies elucidating the molecular  
3 mechanisms contributing to differential responses under common environmental conditions  
4 will be an important next step. Moreover, future studies that expand the investigation of sex-  
5 specific/biased transgenerational plasticity will be also valuable to understand if the effects are  
6 transmitted selectively to the offspring.

7

#### 8 **4.6. Within- and transgenerational plasticity is not influenced by gametophyte kinship**

9 Kelp species are capable of intergametophytic selfing, i.e., mating between a female and a male  
10 gametophyte derived from the same diploid sporophyte (Raimondi et al., 2004; Carney et al.,  
11 2013; Itou et al., 2019), potentially resulting in inbreeding depression. Selfing leads to a variety  
12 of negative fitness effects in *Macrocystis pyrifera* (Raimondi et al., 2004; San Miguel, 2017;  
13 Camus et al., 2021). In contrast, we found no influence of selfing vs outcrossing on female  
14 reproductive success, sporophyte formation or thermal tolerance of offspring sporophytes in *L.*  
15 *pallida*, regardless of the thermal history. However, our study was confined to one relatively  
16 isolated population from the Northern distributional range edge of *L. pallida*, and the costs of  
17 selfing may vary between populations.

18 In isolated kelp populations or in marine forests with sparsely distributed individuals, self-  
19 fertilization may be especially pronounced compared to central populations forming large  
20 forest networks (Barner et al., 2011; Camus et al., 2018). Highly inbred populations have been  
21 shown to have low inbreeding depression (Charlesworth et al., 1990). In *M. pyrifera*, the  
22 negative male-female kinship effect on reproductive output was shown to be weaker in low  
23 latitude marginal populations (Camus et al., 2021). Moreover, inbreeding did not impact the  
24 thermal tolerance of the offspring from patchy populations of the kelp *Postelsia palmaeformis*  
25 (Barner et al., 2011). Populations of *L. pallida* from Northern Namibia, including the one used

1 in this work, are much less diverse than those in the southern range (Assis et al. 2022), thus it  
2 is likely that outcrossing offers only slightly greater heterozygosity than selfing, reducing the  
3 capacity to detect the effects of inbreeding depression against a low diversity background. The  
4 absence of inbreeding depression may be also attributed to genetic ‘purging.’ The accumulated  
5 deleterious alleles in populations that suffered reductions in size may be purged through natural  
6 selection (Barrett & Charlesworth, 1991; Byers & Waller, 1999).

7 Selfing may decrease the potential for genetic adaptations to arise when facing  
8 environmental changes, thus reducing evolutionary potential in the long-term (Barner et al.,  
9 2011). However, short-term plastic responses to environmental changes might not require high  
10 levels of genetic variation (Buckley et al., 2019). Several studies have focused on the effects  
11 of inbreeding on short-term plastic responses to environmental heterogeneity; most show that  
12 plasticity is negatively affected by inbreeding (Campbell et al., 2014; Auld and Relyea 2010).  
13 However, studies focusing on how carry-over plasticity is affected by inbreeding are scarce,  
14 particularly in macroalgae. In our study, the within- and transgenerational plasticity patterns  
15 observed in *L. pallida* did not differ between intergametophytic selfing and outcrossing. Thus,  
16 no relationship was found between mating systems and expression of phenotypic plasticity  
17 within and across generations. Recently, Camus et al. (2021) discovered that inbred and  
18 outcross offspring sporophytes showed no differences in tolerance to heat waves. In the light  
19 of the results observed in this inbred population, it would be interesting to explore whether the  
20 absence of selfing on fitness and phenotypic plasticity might differ among *L. pallida*  
21 populations with different levels of inbreeding.

22 Together, our results suggest that thermal plasticity plays an important role in the response of  
23 this Northern range population of *L. pallida* to environmental change, contributing to their  
24 successful persistence and adaptation. However, it also reveals that these responses are  
25 complex, depending on thermal history, maternal genetics, time of exposure to thermal stress

1 and physiological traits, and such dependency needs to be considered when predicting  
2 organismal and/or population responses to a changing world. We are far from fully  
3 understanding the intricacies and complexity of within- and transgenerational plasticity  
4 responses of kelps to environmental stressors. Thus, future research should investigate the  
5 extent at which phenotypic plasticity could buffer kelp populations with multiple  
6 environmental stressors and their stability across multiple generations.

7

8 Acknowledgements. This study received Portuguese national funds from FCT - Foundation for  
9 Science and Technology through SFRH/BPD/122567/2016 to NM (in transitional norm DL  
10 57/2016/CP1361/CT0039), SFRH/BD/139189/2018 to LB and the project UIDB/04326/2020.  
11 Namibian sampling was funded by the Benguela Current Convention (BCC) project BCLME  
12 III, which received funds from the Global Environment Facility (GEF) and United Nations  
13 Development Program (UNDP).

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7

1 Table 1. Crosses used to evaluate gametophyte reproduction (3 inbred and 6 outcrossed). The  
2 crosses used to evaluate thermal plasticity of microscopic F1 sporophytes are highlighted in  
3 bold (3 inbred 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>Inbred</b>	<b>Outcrossed</b>	Outcrossed
$\text{\textcircled{g}}\text{3}$	Outcrossed	<b>Inbred</b>	<b>Outcrossed</b>
$\text{\textcircled{g}}\text{6}$	<b>Outcrossed</b>	Outcrossed	<b>Inbred</b>

4

5

1   **Table 2.** ANOVA for the effects of strain and temperature on the  $F_v/F_m$  of *Laminaria pallida*  
2   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 × Temperature	5	0.10	0.02	1.01	0.434
Residual	24	0.46	0.02		

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

5

1   **Table 3.** ANOVA for the effects of cross and thermal history (8 and 20°C) on the absolute  
2   growth rate for gametophyte area of *Laminaria pallida* after 6 days in gametogenic conditions.  
3   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		

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

6

1   **Table 4.** ANOVA for the effects of cross and thermal history (8 and 20°C) on the percentage  
 2   of reproductive female gametophytes after 10 days (a), percentage of female gametophytes  
 3   with sporophytes after 28 days (b) and the absolute number of sporophytes per female  
 4   gametophytes after 30 days (c) in gametogenic conditions. The post-hoc results are presented  
 5   in Fig. 4.

<b>Factor</b>		<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
<hr/>						
(a) % Reproductive female						
gamtophytes 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			
(c) Sporophytes/female						
gamtophyte at day 30						
Cross	8	5.40	0.674	15.15	<0.001	
Thermal history	1	3.55	3.55	79.74	<0.001	
Cross × Thermal history	8	3.25	0.41	9.11	<0.001	
Residual	53	2.36	0.05			

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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 5.** ANOVA for the effects of cross, temperature (8, 14, 20 and 23°C) and thermal history  
 2   on the normalized density of microscopic sporophytes of *Laminaria pallida* after 16 days. The  
 3   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		

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

6

1   **Table 6.** PERMANOVA for the effects of cross, temperature (8, 14, 20 and 23°C) and thermal  
 2   history on the photosynthetic efficiency of *Laminaria pallida* sporophytes after 16 days. The  
 3   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>
<b>(a) Normalised F<sub>v</sub>/F<sub>m</sub></b>					
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>
history					
Residual	144	2.30	0.02		
<b>(a) Normalised rETRmax</b>					
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>
history					
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

1   **Table 7.** ANOVA for the effects of cross, temperature (8, 14, 20 and 23°C) and thermal history  
 2   on the absolute growth rate of sporophytes of *Laminaria pallida* after two time periods, (a) 0 -  
 3   8 days and (b) 8 - 16 days. The post-hoc results are presented in Figs. 7 and 8 and Table S2.

<b>Factor</b>	<b>df</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
<b>(a) Absolute growth rate, d0-d8</b>					
Cross	5	7306.75	1461.35	59.26	<b>&lt;0.001</b>
Temperature	3	5131.29	1710.43	69.36	<b>&lt;0.001</b>
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 x Thermal history	15	930.61	62.04	2.52	<b>0.002</b>
history					
Residual	144	3550.99	24.66		
<b>(b) Absolute growth rate, d8-d16</b>					
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 x Thermal history	15	3725.04	248.34	1.44	0.136
history					

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Residual	144	24818.65	172.35
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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

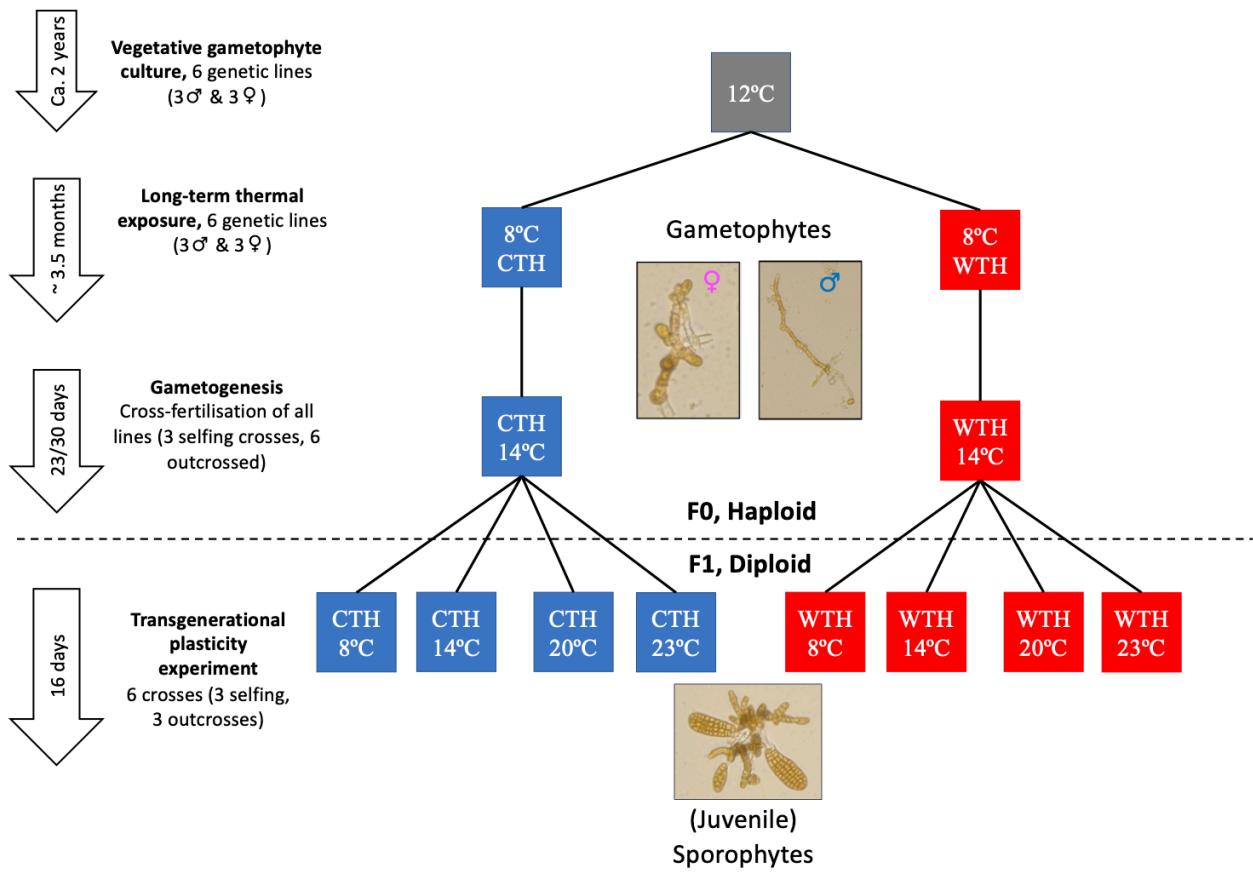
1   **Table S1.** Summary of the number of significant differences between thermal histories (8°C:  
 2   CTH and 20°C: WTH) per experimental temperature (8, 14, 20 and 23°C) in the photosynthetic  
 3   parameters of *Laminaria pallida* sporophytes.

<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  
 7   **Table S2.** Summary of the number of the significant differences between thermal histories  
 8   (8°C: CTH and 20°C: WTH) per experimental temperature (8, 14, 20 and 23°C) in the absolute  
 9   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

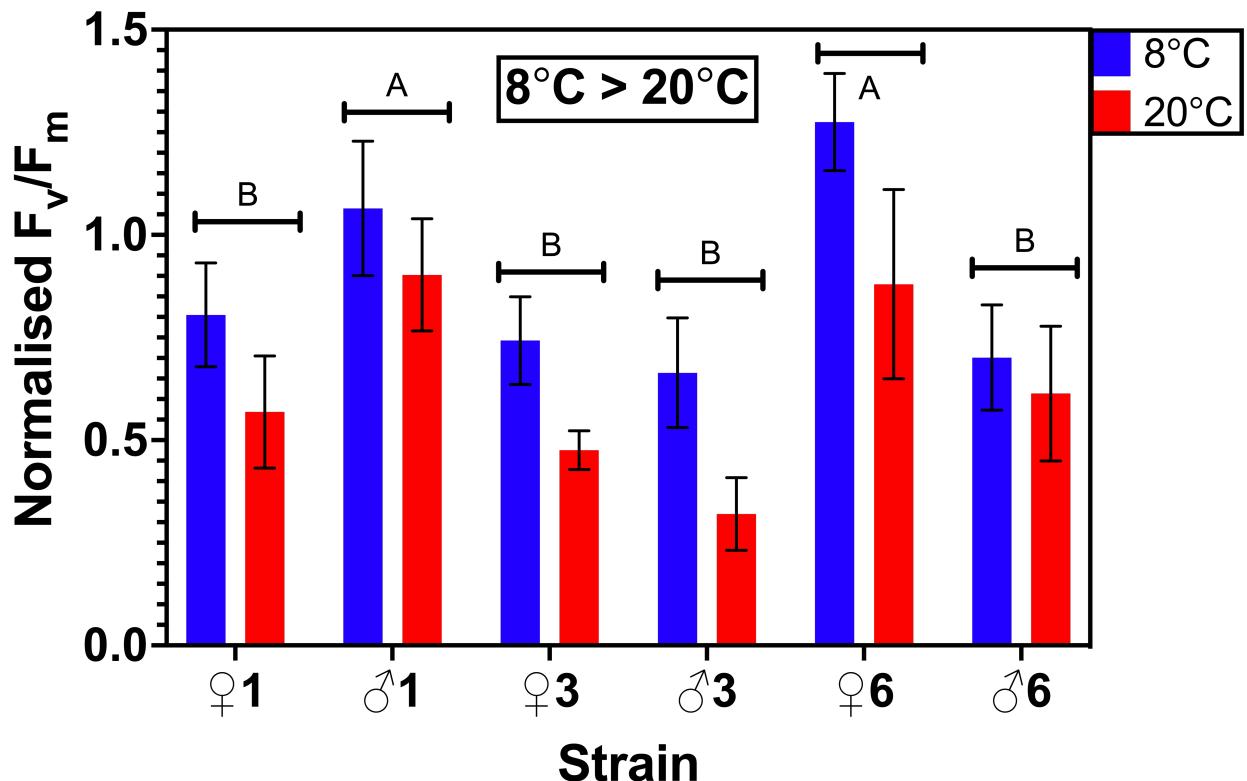
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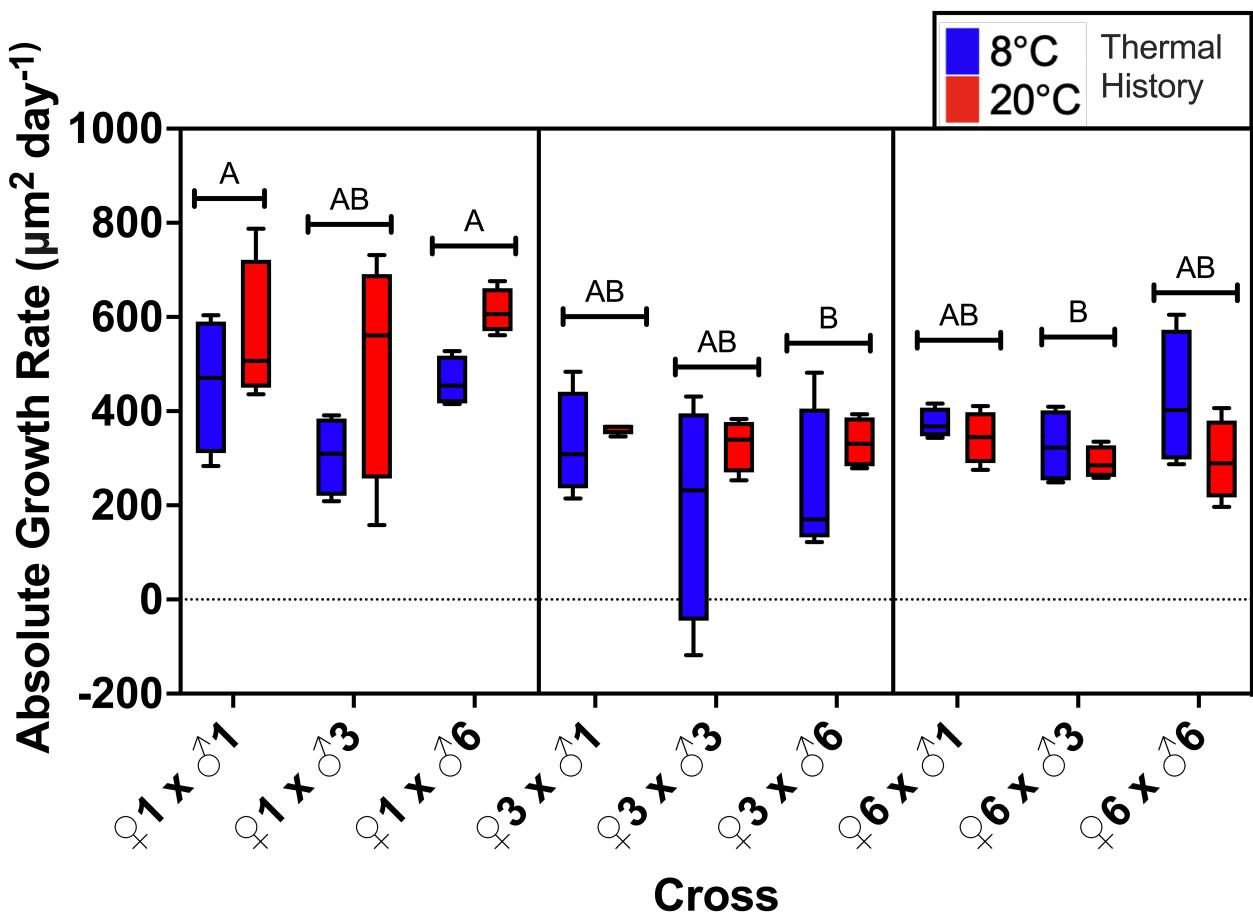
1

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 inbred 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).

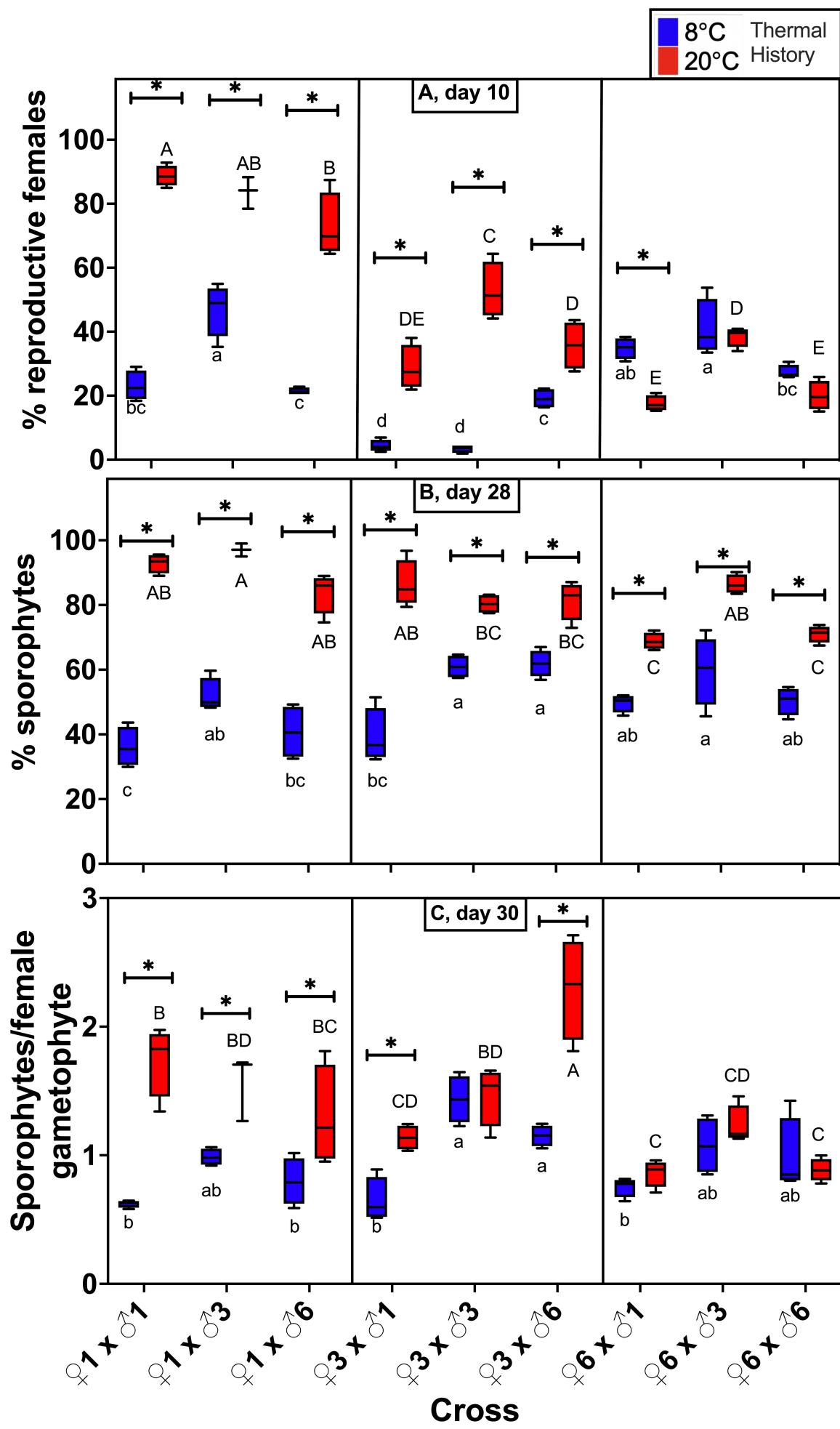
11



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 Please note that  $F_v/F_m$  values were normalized to the respective initial value for each strain.  
5 Bar plots with mean and error bars with standard deviation (n = 4). Different letters indicate  
6 significant differences between strains. See Table 2 for statistics.  
7

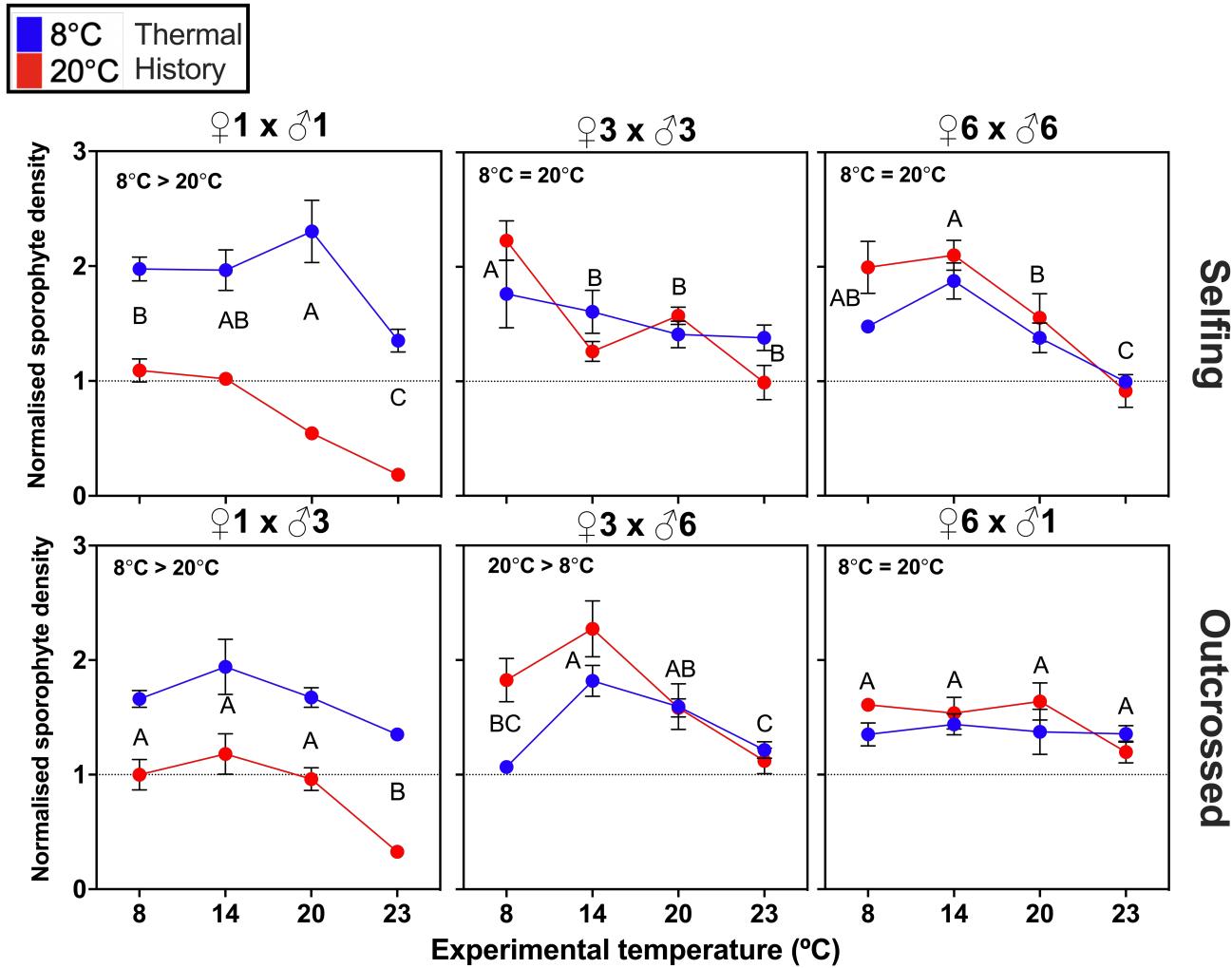


1  
2 **Figure 3: Effect of thermal history on the absolute growth rate of gametophytes from**  
3 **different *L. pallida* crosses after 6 days under gametogenic conditions.** Box plots with  
4 median, boxes for 25<sup>th</sup> and 75<sup>th</sup> percentiles and whiskers indicating min and max values (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

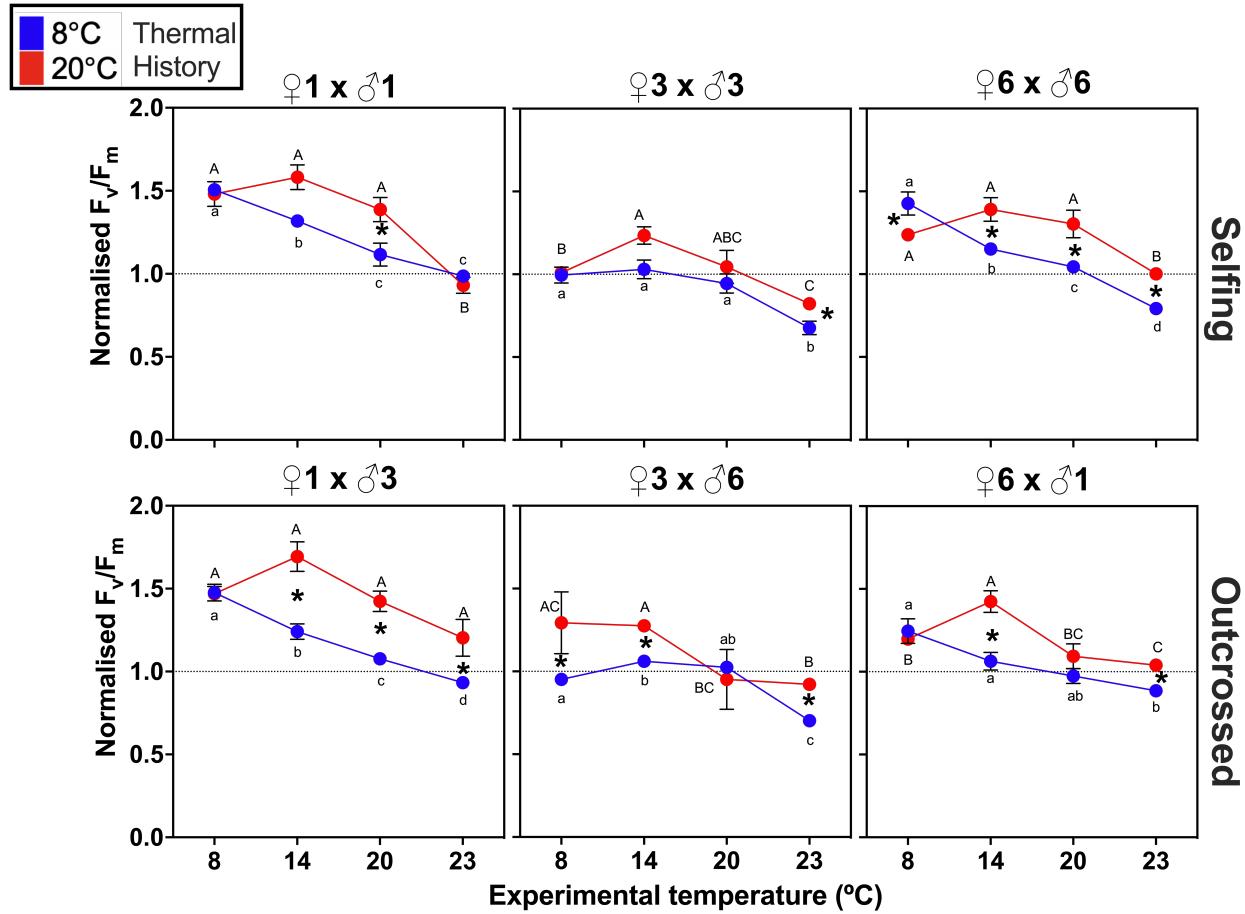


1   **Figure 4: Effect of thermal history on the reproductive success of different *L. pallida***  
2   **crosses in gametogenic conditions.** (A) Percentage of reproductive female gametophytes (egg  
3   released and sporophytes formed) after 10 days. (B) Percentage of female gametophytes with  
4   sporophyte(s) after 28 days. (C) Absolute number of sporophytes per female gametophyte after  
5   30 days. Box plots with median, boxes for 25<sup>th</sup> and 75<sup>th</sup> percentiles and whiskers indicating  
6   min and max values (n = 4). Panels separate crosses with different females. \*Indicates a  
7   significant difference between thermal histories per cross. For each thermal history, different  
8   letters indicate significant differences between crosses ( $p < 0.05$ , uppercase letters for the 20°C  
9   thermal history and lowercase for the 8°C thermal history). See Table 4 for statistics.

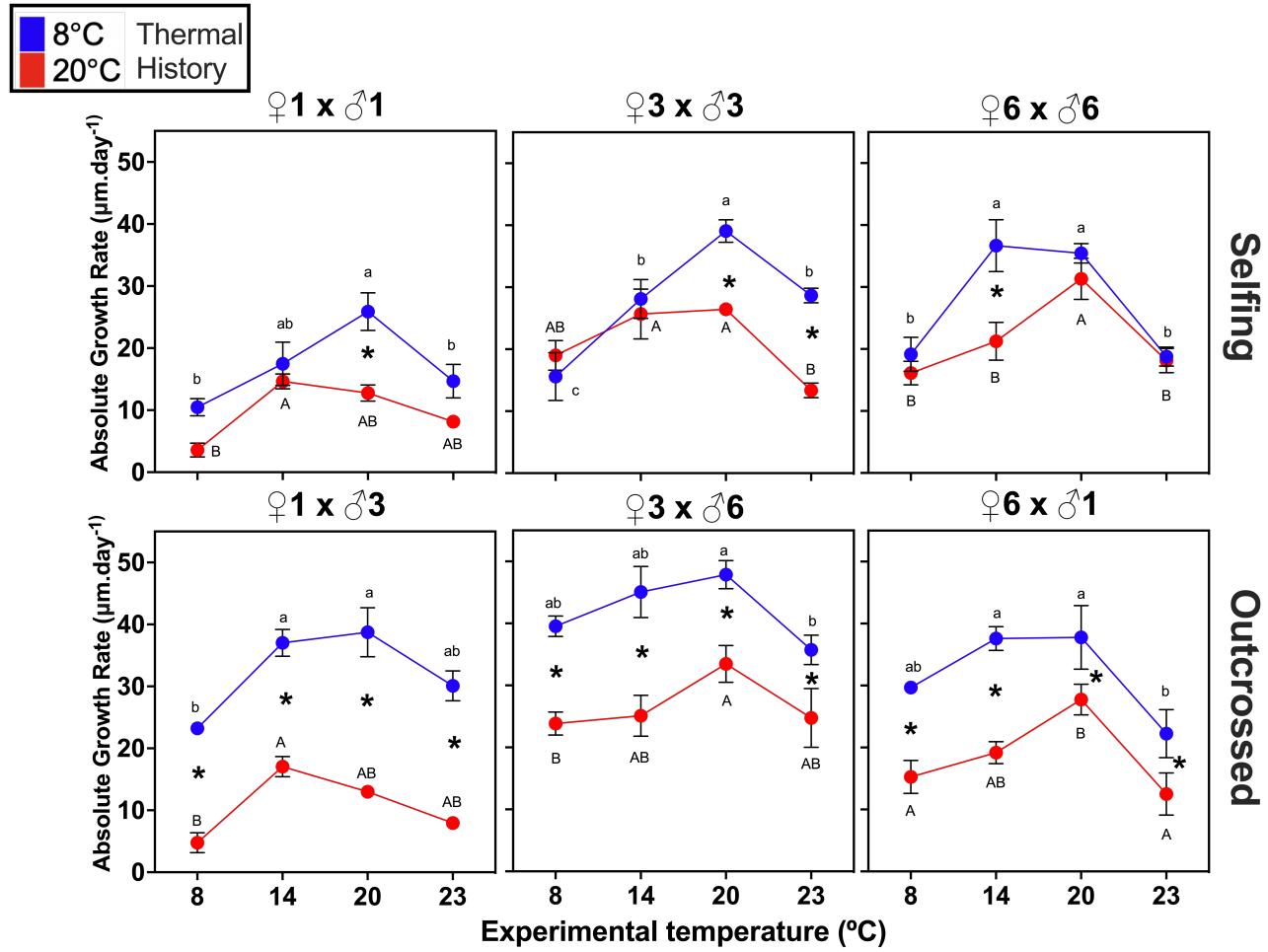
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1  
2 **Figure 5: Effect of parental thermal history on the sporophyte density of different *L.*  
3 *pallida* crosses after 16 days in experimental temperatures (8°C, 14°C, 20°C and 23°C).**  
4 Connected mean plots with standard error of the mean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



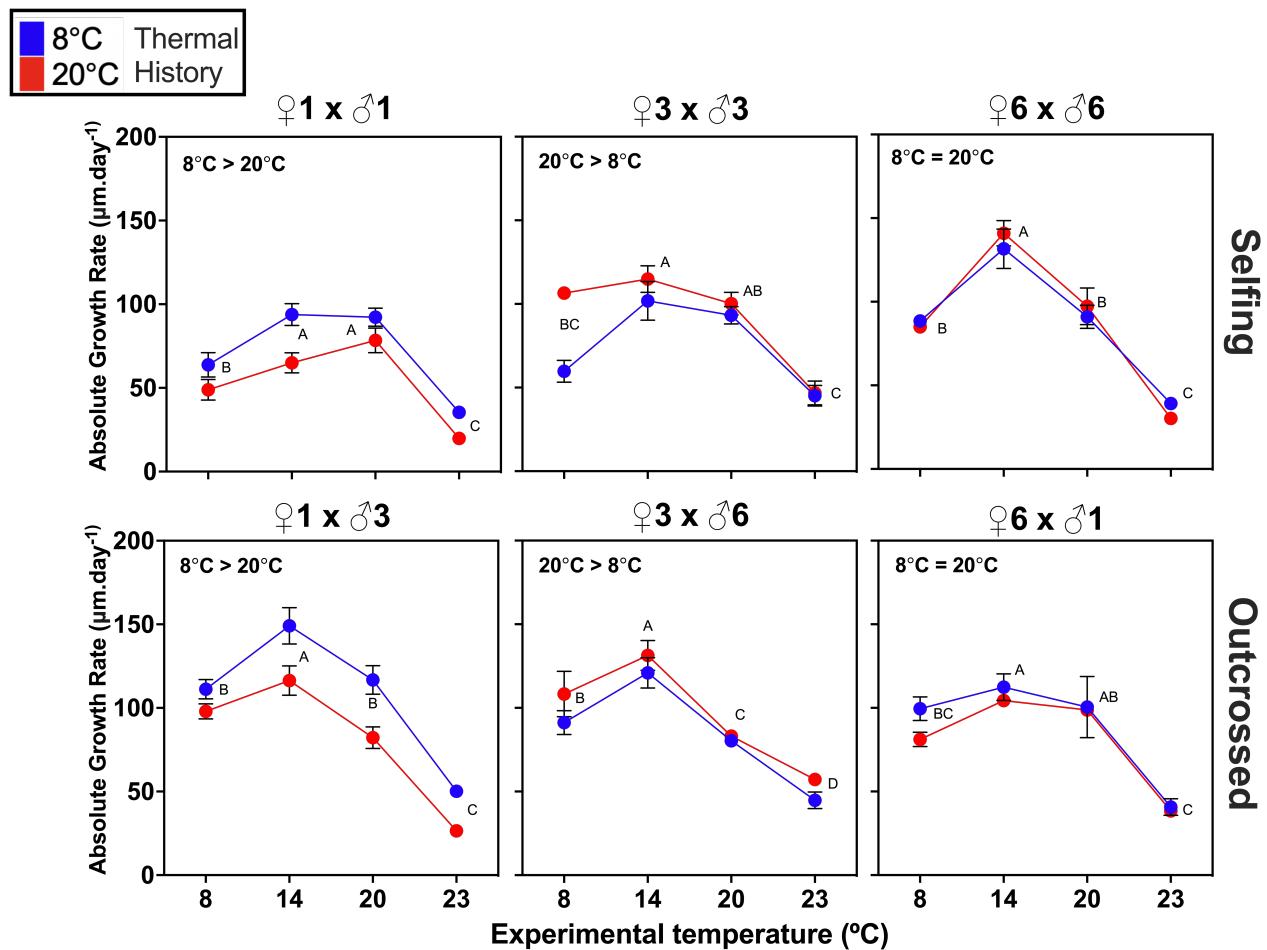
1  
2 **Figure 6: Effect on parental thermal history on the sporophyte maximum photosynthetic  
3 yield ( $F_v/F_m$ ) of different *L. pallida* crosses after 16 days in experimental temperatures  
4 (8°C, 14°C, 20°C and 23°C). Connected mean plots with standard error of the mean (n = 4).**  
5 Each plot corresponds to a cross of parental gametophytes. \* 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 6 for statistics.  
10



1

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.

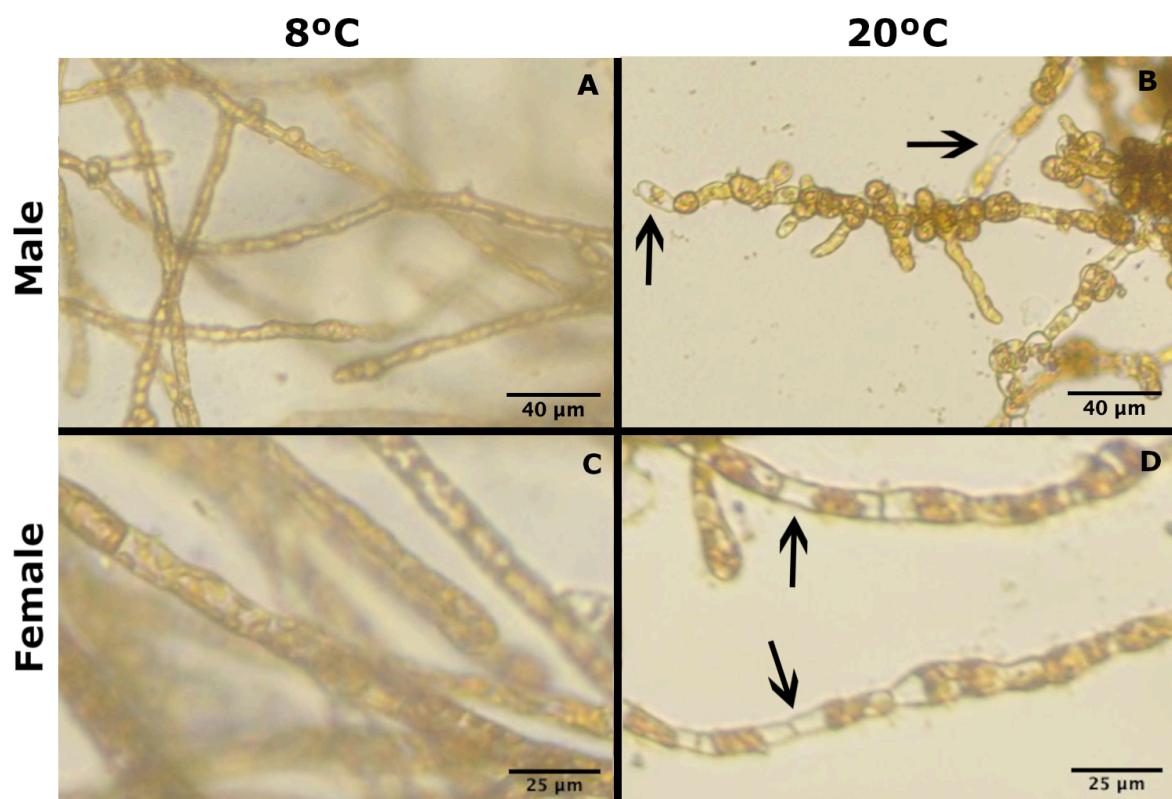
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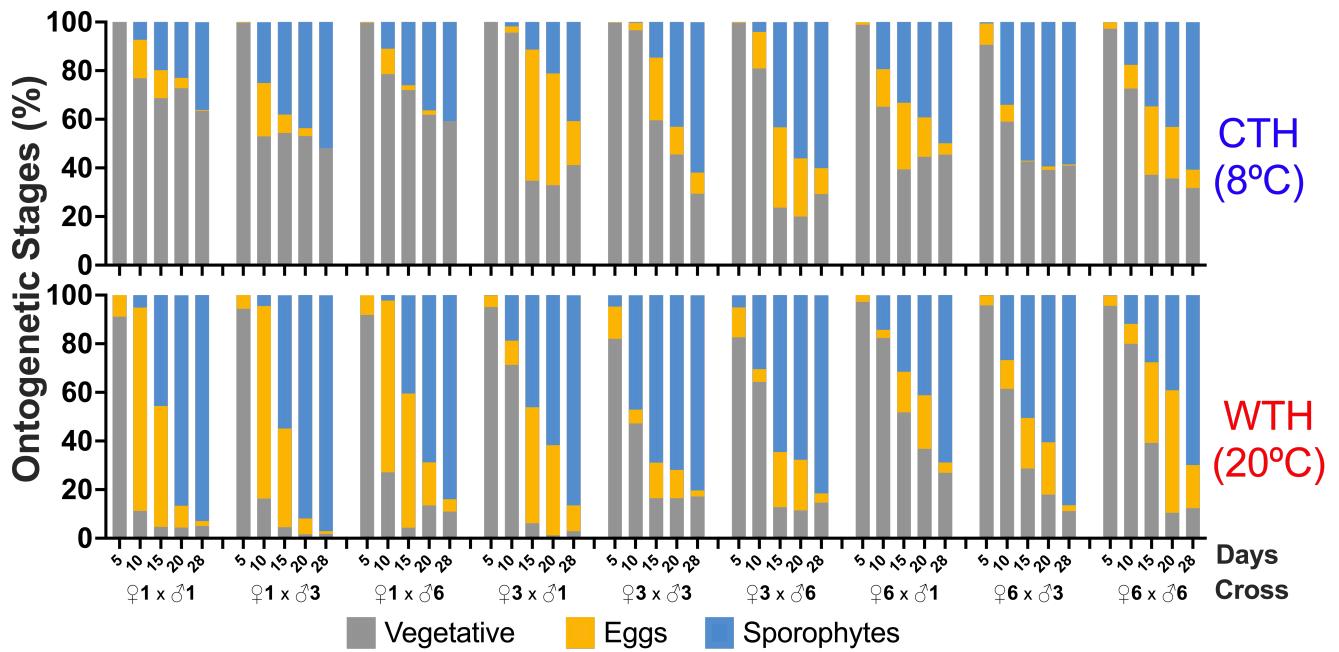
1

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



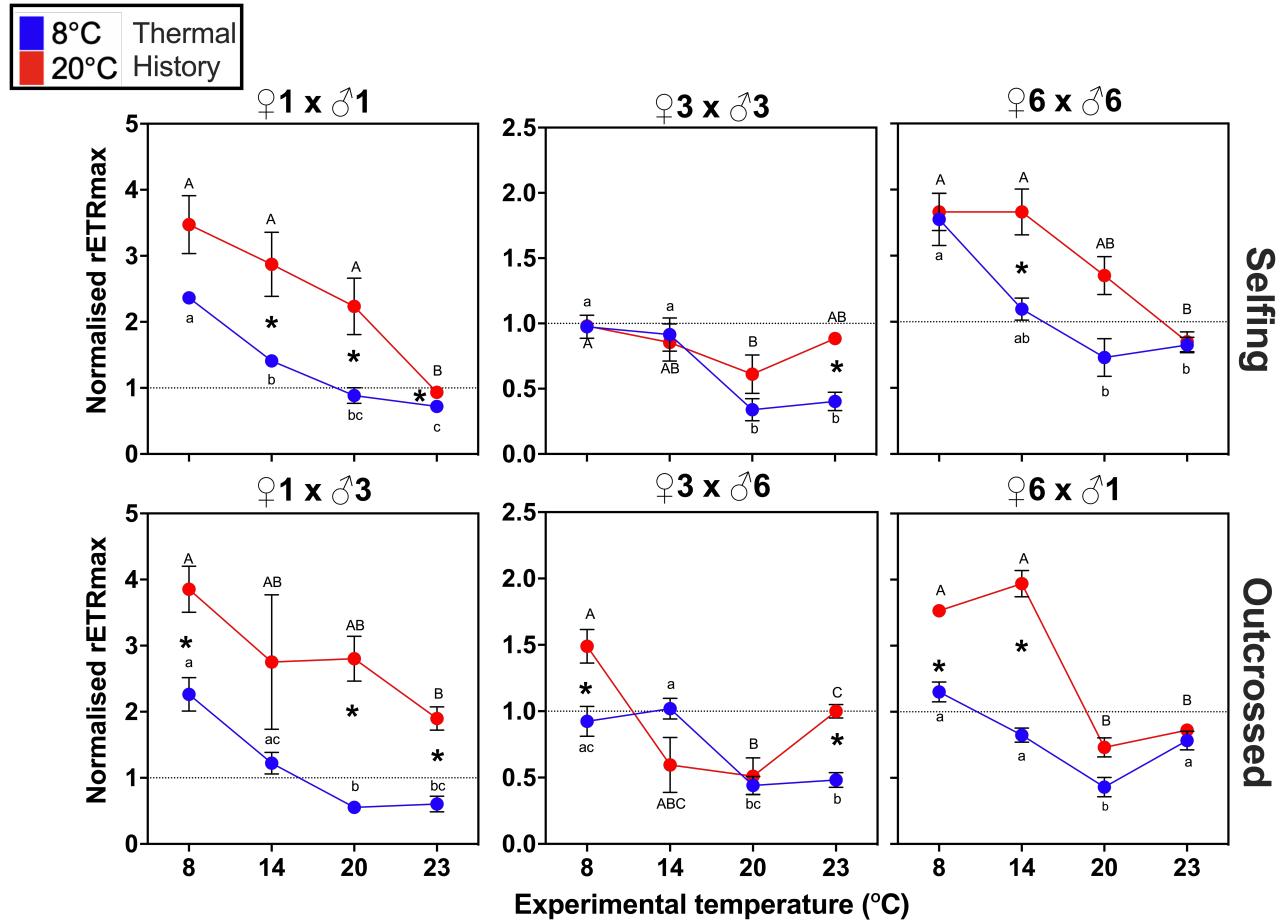
1  
2 **Figure S1: Gametophytes of *Laminaria pallida* after ~3.5 months of temperature exposure**  
3 **at 8°C and 20°C.** A, B. Male gametophyte cells. C, D. Female gametophyte cells. Arrows  
4 indicate dead or damaged cells.  
5



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.  
 10