

1 **Effects of thermal history on reproductive conditioning and transgenerational plasticity**
2 **in the 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
6 conditioning and transgenerational plasticity in the warm-temperate kelp *Laminaria pallida*.
7 Our results show that a warm temperature during the vegetative growth of gametophytes
8 improved subsequent reproductive success under optimum compared to cold conditions.
9 Transgenerational plasticity was also evident; thermal tolerance of microscopic F1 sporophytes
10 was modulated by the thermal history of the parental gametophytes, but the effects were
11 complex varying with the physiological trait and the exposure time. Genetic lines differed in
12 their capacity for reproductive conditioning and plasticity, with the direction and strength of
13 responses more dependent on the female parental genome. This genetic variation suggests that
14 thermal plasticity can potentially evolve under ocean warming. Our results indicated that the
15 degree of gametophyte kinship (inbreeding versus outcrossing) does not influence the thermal
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 reproductive conditioning and
19 transgenerational plasticity can play an important role in *L. pallida* adaptation to warm habitats,
20 highlighting the importance of incorporating thermal history effects in physiological studies to
21 accurately predict the vulnerability of populations and species to future warming climates.
22 Further understanding of kelp phenotypic plasticity will help to secure sustainable seaweed
23 aquaculture and population restoration strategies through the establishment of priming
24 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 combinations of phenotypic plasticity
6 and genetic adaptation (Donelson et al., 2019). Sessile organisms are particularly vulnerable to
7 climate change as natural distribution shifts and adaptation via natural selection may be too
8 slow in the face of rapidly changing conditions (Atkins & Travis, 2010), posing a risk of range
9 contraction and local extirpations. Consequently, phenotypic plasticity is critical in increasing
10 the 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). As a genetically driven mechanism,
15 it varies between individuals and can be a target for selection. Phenotypic plasticity can occur
16 both within a generation (within-generational) and across generations (transgenerational).
17 Thus, understanding the interactions and contributions of within- and transgenerational
18 plasticity to the phenotypic response is important for the prediction of species adaptation to
19 changing environments (Auge et al., 2017).

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

1 to overcome many different environmental stressors (Galloway & Etterson, 2007; Herman &
2 Sultan, 2011). However, TGP can also be neutral or even maladaptive to the performance of
3 species or populations in a changing world and has the potential to build-up across generations
4 and 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; Oliver et al., 2018; Smale, 2020), providing natural protection against coastal erosion
17 and acting as significant long-term carbon sinks (Buchholz et al., 2012). Kelp forests also have
18 a high economic value providing a wide range of ecosystem goods and services (e.g., Bennet
19 et al., 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, 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, 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 and more fragmented than southern ones and are usually

1 exposed to warmer temperatures (Rothman et al., 2015). Recent research suggests long-term
2 stability of *L. pallida* throughout its current distributional range (Assis et al., 2022), but the
3 complexity of the regional Benguela current makes precise predictions difficult (Kainge et al.,
4 2020).

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

18

19 **2. MATERIALS AND METHODS**

20 **2.1. Experimental design**

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

1 sporophytes were reared for 16 days at a range of temperatures (8-23°C) to test the effects of
2 parental thermal history on their thermal tolerance. Several gametophyte strains from a single
3 population were used to assess genetic variation in plasticity.

4 **2.2. Algal material**

5 Three mature sporophytes of *Laminaria pallida* were sampled from Swakopmund, Namibia
6 (22.672 S, 14.522 E), in the Northern distributional range of the species in July 2019. There,
7 minimum surface seawater temperatures of 12°C are recorded during winter, while it reaches
8 22°C during the Austral summer (Demarcq et al., 2003), close to the upper survival temperature
9 (23°C) of *L. pallida* sporophytes (Martins et al., 2019). Sori were cleaned and meiospores from
10 each sporophyte were released separately in sterile seawater. After spore germination, separate
11 male and female gametophyte stock cultures were established for each individual (culture
12 numbers: 1, 3 and 6) and maintained in a vegetative state in sterile half-strength Provasoli
13 enriched seawater (PES; Provasoli, 1968) at 12°C under 3 µmol photons m⁻² s⁻¹ of red light and
14 16h:8h light:dark (L:D) cycle in a climate-controlled chamber (Fitoclima S600, Aralab, Lisbon,
15 Portugal). Sterile artificial seawater (Tropic Marin Sea Salt, Wartenberg, Germany) with a
16 salinity of 34 ± 1 ppm was used for maintenance and all experiments. The culture medium was
17 changed monthly, until the beginning of the 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⁻² 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⁻¹. 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⁻². 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⁻² s⁻¹ of red light in a 16h:8h L:D cycle. After this period the gametophytes
20 were transferred to 17 µmol photons m⁻² s⁻¹ 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 reproductive gametophytes (egg released or sporophyte attached) on day 10, since at
23 least one cross showed over 80% of reproductive females. Reproductive success was evaluated
24 by considering the percentage of female gametophytes with sporophytes after 28 days and the
25 absolute number of sporophytes per female gametophyte after 30 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 inbred 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⁻¹. 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⁻² s⁻¹ 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 and remaining
7 gametophytes. A FluorPen FP 110 (PSI, Drásov, Czech Republic) was used to measure the
8 maximum photosynthetic yield of PSII (F_v/F_m) as well as the response to a light curve (Light
9 curve 1, Flash pulse: 20%, Super pulse: 40%, Actinic pulse: 18 μmol) in each replicate. The
10 light curve response was used to calculate the relative maximum electron transport rate
11 (rETRmax) using the Phytotools package in R software (R Core Team, 2021; Silsbe & Malkin,
12 2015). Sporophytes were dark acclimated for five minutes before the measurements. Data was
13 normalised (divided by the mean values at day 0) to account for significant differences initially
14 between sporophytes from different crosses, allowing comparisons between crosses.

15

16 **2.5.c. Sporophyte growth**

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

1 **2.6. Statistical Analysis**

2 Data was analysed using SPSS 27 software (IBM corp., Armonk, NY, USA) and the
3 PERMANOVA module of Primer 6 software (Anderson, 2001; McArdle & Anderson, 2001).
4 Data was tested for normality within groups using the Shapiro-Wilk test and homoscedasticity
5 using Levene's test in SPSS. The normalised F_v/F_m of gametophytes was analysed under a two-
6 factor ANOVA (fixed factors: strain and temperature). Gametophyte absolute growth rate
7 (square root transformed), percentage of reproductive females after 10 days, percentage of
8 female gametophytes with sporophytes after 28 days and absolute number of sporophytes per
9 female gametophyte data were also analysed under a two-factor ANOVA (fixed factors: cross
10 and thermal history). Sporophyte density (log-transformed) and sporophyte length-AGR data
11 were analysed under a three-factor ANOVA (fixed factors: cross, thermal history and
12 experimental temperature). Post-hoc Tukey tests with Bonferroni corrections ($FDR \leq 0.05$)
13 were conducted to determine differences between treatments when significant main effects or
14 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 health 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 reproductive females (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 reproductive females occurred in the crosses ♀3 ×
3 ♂1 and ♀3 × ♂3. In WTH gametophytes, female fertility was significantly higher in crosses
4 with ♀1 compared with all other crosses.

5 For all crosses, WTH resulted in significantly higher reproductive success than CTH ones.
6 The percentage of female gametophytes with sporophytes after 28 days showed a significant
7 cross × thermal history interaction (Table 4b, Fig. 4B). In the WTH gametophytes, the
8 relative sporophyte presence was higher in the cross ♀1 × ♂3 (97%) than in two of the
9 crosses with ♀3 (♀3 × ♂3 and ♀3 × ♂6; mean value of 81%) and with ♀6 (♀6 × ♂1 and ♀6
10 × ♂6; mean value of 70%). On the other hand, the crosses ♀3 × ♂3, ♀3 × ♂6 and ♀6 × ♂3
11 (mean value of 61%) with CTH developed a higher proportion of sporophytes than ♀1 × ♂1,
12 ♀1 × ♂6 and ♀3 × ♂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 (sporophyte production)(reproductive conditioning); (2) Strain-specific
16 variation in within-generational plasticity was observed as gametophyte strains ♀1 and ♀3
17 were in general more prone to the effect of thermal history than ♀6; 3) Male gametophytes
18 affect female gametophyte reproduction, as each female strain showed different reproductive
19 success when crossed with distinct male strains.

20

21 **3.3. Transgenerational effects of thermal history**

22 3.3.a. Sporophyte density

23 Sporophyte densities increased in the ♀1 crosses with CTH over the 16 days (normalised
24 density > 1) independent of the experimental temperature, indicating ongoing gametophyte
25 reproduction and sporophyte formation (Fig. 5). Similarly, increases in sporophyte density

were also observed in the remaining crosses at 8, 14 and 20°C, regardless of the parental thermal history. At the higher temperature (23°C), further maturation seems to be prevented, and the stable sporophyte densities reflect survival rather than additional development in these crosses. In the two WTH ♀1 crosses, sporophyte densities remained stable (normalised density ≈ 1) at the lowest temperatures (8 and 14°C), but decreased at higher temperatures, particularly at 23°C, indicating sporophyte mortality.

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

15

16 3.3.b. Sporophyte photosynthetic responses

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

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

1 in the CTH sporophytes. At the highest temperature of 23°C, F_v/F_m significantly decreased
2 compared to all the other temperatures in the sporophytes from both thermal histories (1.4-fold
3 overall). For both thermal histories, rETRmax showed a decreasing trend at $\geq 20^\circ\text{C}$ compared
4 with the lower temperatures (Fig. S3).

5

6 **3.3.c. Sporophyte growth**

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

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

23

24 **4. DISCUSSION**

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

21

22 **4.1. Decreased gametophyte health under warm seawater temperature**

23 Although *L. pallida* sporophytes are reproductive year-round, the peak is mainly at the end of
24 the austral summer (March, Dieckmann, 1980), when surface temperatures are still warm (17-
25 21°C in Swakopmund, seatemperature.org). Spores that settle on rocky substrate and develop

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

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

18

19 **4.2. Reproductive conditioning: warm thermal history promotes gametogenesis and**
20 **female reproductive success**

21 We found that gametophyte thermal history impacts female gametophyte reproduction,
22 revealing reproductive conditioning in *L. pallida*. Although the development under warm
23 seawater temperature (20°C) reduced the gametophyte physiological performance, it promoted
24 the speed of gametogenesis and the reproductive success under optimal conditions. Similarly,
25 in the kelp *Alaria esculenta* faster gametogenesis in WTH (22°C) gametophytes was suggested

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

7 The WTH promotion of *L. pallida* gametogenesis and sporophyte formation is consistent with
8 its seasonal reproductive pattern in the native environment. Spores release at the end of the
9 summer (Rothman et al., 2015) suggests that gametophyte development occurs seasonally
10 under high summer/autumn seawater temperatures, while sporophyte formation occurs during
11 lower winter temperatures (Dieckmann, 1978). Reproduction in Laminariales is known to be
12 associated with seasonal environmental cues, including changing seawater temperature,
13 daylength or irradiance (Bettignies et al. 2018; Martins et al., 2017; Kain 1989). The warm
14 thermal environment to which gametophytes were exposed may have acted as a seasonal
15 thermal cue, preconditioning gametophytes for reproduction when cooler conditions followed.
16 In other brown algae, like fucoids, gametogenesis initiates in response to short days (Bäck et
17 al., 1991; Berger et al., 2001), whereas water movements and lunar or tidal cycles are key
18 proximal signals in gamete release (Monteiro et al. 2016; Pearson et al., 1998; Pearson &
19 Serrão, 2006; Serrao et al., 1996). In some Arctic kelp species, gametogenesis occurs only
20 under short days (<8 h light) and low seawater temperatures (Wiencke & tom Dieck, 1989).
21 The warm-temperate kelp *Ecklonia radiata* produces the healthiest gametophytes when days
22 are long to promote germination and growth, but gametogenesis and sporophyte production
23 occurs later when daylengths are shorter and water temperature declines (Mohring et al., 2013).
24 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 Additionally, heat-stress is known to accelerate or even induce reproduction in plants (reviewed
4 by Takeno, 2016) and seagrasses (Blok et al., 2018; Marín-Guirao et al., 2019; Ruiz et al.,
5 2018), and enhanced the production of new gametophytic thalli accelerating the asexual
6 reproduction in the red alga *Pyropia yezoensis* (Suda & Mikami, 2020). Such stress-related
7 effects may add to the seasonal conditioning in promoting reproduction in WTH gametophytes
8 of *L. pallida*.

9

10 **4.3 Female gametophyte reproduction is shaped by the male strain**

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

25

1 **4.4. Transgenerational effects on the thermal tolerance of microscopic sporophytes**

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

20 Although studies on thermal plasticity across generations have recently increased for marine
21 organisms (McRae et al., 2021; Shama et al., 2016), the temporal effects on offspring
22 performance are still unclear. In our study, sporophyte growth responses varied over exposure
23 time to a range of temperatures; sporophytes from CTH grew significantly more than WTH
24 during the first 8 days, particularly at mild to warm temperatures (14-23°C). This may represent
25 a potential “silver-spoon” parental effect (Baker et al., 2019; Germain et al., 2019), which

describes a physiological advantage for individuals whose parents had access to abundant resources. Similarly, in the cold-temperate kelp *L. digitata* the growth of early sporophytes at extreme temperatures (0°C and 20°C) was improved by a cold parental thermal history (5°C; Gauci, 2020). Interestingly, the “silver spoon” parental effect disappeared over time (from day 8 to day 16) and thermal plasticity dependent on the maternal line become evident. In the offspring from maternal line ♀1 growth was enhanced by a parental CTH, while the WTH sporophytes from maternal line ♀3 grew more than those from CTH. On the other hand, parental thermal history had no influence on the growth of the offspring from maternal line ♀6. These results suggest a more complex picture of the effects of thermal history on the offspring performance of this warm temperate kelp species that are dependent of the temperature exposure duration in the offspring.

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

24

1 **4.5. Genetic variation in within- and transgenerational plasticity: via female or maternal**
2 **effects**

3 Genetic variation in reproductive conditioning and transgenerational plasticity for thermal
4 tolerance was demonstrated for *L. pallida*. Within a generation, the phenotypic plasticity
5 response of gametophytes to thermal history was dependent on the female genetics. Crosses
6 with ♀1 and ♀3 showed within-generational plasticity for reproductive success, with a WTH
7 enhancing fertility and offspring output. However, thermal history had less influence in crosses
8 with ♀6, particularly on the production of sporophytes. Similarly, the effect intensity differed
9 between crosses with ♀1 and ♀3 (stronger in ♀1 crosses with respect to reproductive success
10 between WTH and CTH gametophytes). These variations in reproductive plasticity within a
11 generation may be linked to the aptitude to form and retain epigenetic markers, e.g., DNA
12 methylation or histone modification. Genetic variation in plasticity has been extensively
13 described in plants (Sultan et al., 2009; Vu et al., 2015) and was recently shown in kelp species
14 (Alsuwaiyan et al., 2021; Mabin et al., 2019; Liesner et al., 2020). Alsuwaiyan et al. (2021)
15 found that haploid and diploid life stages of *Ecklonia radiata* genotypes differed in their
16 susceptibility to thermal stress. Similarly, in *Laminaria digitata* variation in thermal plasticity
17 was reported among genetic lines within a North Sea population (Liesner et al., 2020).
18 Similarly, the direction and strength of the transgenerational plasticity of offspring sporophytes
19 was affected by the maternal genome or epigenome. Sporophyte mortality was only observed
20 in ♀1 crosses with a parental WTH when exposed to the highest temperature (23°C). Moreover,
21 different transgenerational effects on sporophyte growth rates (from day 8 to day 16 of thermal
22 exposure) were observed for each maternal line, suggesting a strong maternal effect on the
23 aptitude for transgenerational plasticity. These results suggest that epigenetic mechanisms are
24 retained more during maternal than paternal gametogenesis. Maternal effects on
25 transgenerational plasticity have been observed in a variety of taxa (e.g., Galloway & Etterson,

1 2007; Marshall, 2008; Shama et al., 2014) and are considered the norm, as females have more
2 ways to impact offspring development (e.g., egg provisioning, transferable maternal material
3 such as mRNA and heritable heat-shock proteins). Evidence of maternal effects on offspring
4 performance also exist in Laminariales (Mabin et al., 2019; Martins et al., 2019; tom Dieck &
5 de Oliveira, 1993; Zhang et al., 2011). For example, in *E. radiata* it was proposed that maternal
6 effects were associated with the gametophyte morphological variation to different temperature
7 and light levels (Mabin et al. 2019). Moreover, thermal responses of interspecific hybrids
8 between *L. digitata* and *L. pallida* revealed that female parents are more important in
9 determining the offspring phenotype than male parents (Martins et al., 2019). Similarly, the
10 female parent was responsible for the sensitivity to low temperature in hybrids between *L.*
11 *pallida* and *L. abyssalis* (tom Dieck & de Oliveira, 1993).

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

17 Our results highlight the importance of considering the degree of genetic variation that is
18 involved in kelp within- and transgenerational plasticity. Studies elucidating the molecular
19 mechanisms contributing to differential responses under common environmental conditions
20 will be an important next step. Moreover, future studies that expand the investigation of sex-
21 specific/biased transgenerational plasticity will be also valuable to understand if the effects are
22 transmitted selectively to subsequent gametophyte generations.

23

1 **4.6. Within- and transgenerational plasticity is not influenced by gametophyte kinship**
2 Kelp species are capable of intergametophytic selfing, i.e., mating between a female and a male
3 gametophyte derived from the same diploid sporophyte (Raimondi et al., 2004; Carney et al.,
4 2013; Itou et al., 2019), potentially resulting in inbreeding depression. Selfing leads to a variety
5 of negative fitness effects in *Macrocystis pyrifera* (Raimondi et al., 2004; San Miguel, 2017;
6 Camus et al., 2021). In contrast, we found no influence of selfing vs outcrossing on female
7 reproductive success, sporophyte formation or thermal tolerance of offspring sporophytes in *L.*
8 *pallida*, regardless of the thermal history. However, our study was confined to one relatively
9 isolated population with low genetic diversity (Assis et al., 2022), and the costs of selfing may
10 vary between populations.

11 Remote and patchy populations of several kelp species tend to suffer less from inbreeding
12 (*Postelsia palmaeformis*: Barner et al., 2011; *M. pyrifera*: Camus et al., 2021) than dense
13 central forest networks (Barner et al., 2011; Camus et al., 2018). In isolated populations, it is
14 likely that outcrossing offers only slightly greater heterozygosity than selfing, reducing the
15 capacity to detect the effects of inbreeding depression against a low diversity background. The
16 absence of inbreeding depression may be also attributed to genetic ‘purging.’ The accumulated
17 deleterious alleles in populations that suffered reductions in size may be purged through natural
18 selection (Barrett & Charlesworth, 1991; Byers & Waller, 1999).

19 While
20 Selfing may decrease the potential for genetic adaptations to arise when facing environmental
21 changes, thus reducing evolutionary potential in the long-term (Barner et al., 2011). However,
22 our results suggest that it does not reduce short-term plastic responses to environmental
23 changes, even across generations. Additionally, Camus et al. (2021) discovered that inbred
24 and outcross offspring sporophytes showed no differences in tolerance to heat waves. On the
25 other hand, several studies have shown that short-term plasticity is negatively affected by

1 inbreeding (Campbell et al., 2014; Auld and Relyea 2010). To better understand potential
2 relationships between mating systems and expression of phenotypic plasticity, it would be
3 interesting to explore whether the absence of selfing on fitness and phenotypic plasticity differs
4 among *L. pallida* populations with different levels of inbreeding.

5

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

16

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- 15

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).

Strain	♀1	♀3	♀6
♂1	Inbred	Outcrossed	Outcrossed
♂3	Outcrossed	Inbred	Outcrossed
♂6	Outcrossed	Outcrossed	Inbred

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

Factor	df	SS	MS	F	P
Strain	5	1.56	0.31	16.33	<0.001
Temperature	1	0.55	0.55	28.93	<0.001
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.

Factor	df	SS	MS	F	P
Cross	8	309.2	38.63	4.66	<0.001
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.

Factor		df	SS	MS	F	P
<hr/>						
(a) % Reproductive female gametophytes 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 gametophyte 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			

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.

Factor	df	SS	MS	F	P
Cross	5	2.62	0.52	6.55	<0.001
Temperature	3	11.57	3.86	48.20	<0.001
Thermal history	1	2.63	2.63	32.90	<0.001
Cross × Temperature	15	4.74	0.32	3.95	<0.001
Cross × Thermal history	5	14.74	2.95	36.84	<0.001
Temperature × Thermal history	3	1.99	0.67	1.42	<0.001
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.

Factor	df	SS	MS	Pseudo - F	P(perm)
(a) Normalised F_v/F_m					
Cross	5	3.09	0.62	38.63	0.0001
Temperature	3	4.53	1.51	94.49	0.0001
Thermal history	1	1.20	1.20	74.90	0.0001
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	0.0002
Cross × Temperature × Thermal history	15	0.63	0.04	2.64	0.0016
history					
Residual	144	2.30	0.02		
(a) Normalised rETRmax					
Cross	5	44.89	8.98	44.45	0.0001
Temperature	3	33.35	11.12	55.04	0.0001
Thermal history	1	20.81	20.81	103.03	0.0001
Cross × Temperature	15	12.16	0.81	4.01	0.0001
Cross × Thermal history	5	13.77	2.75	13.64	0.0001
Temperature × Thermal history	3	0.93	0.31	1.55	0.2049
Cross × Temperature × Thermal history	15	5.76	0.38	1.90	0.0264
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.

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	<0.001
Cross × Temperature	15	624.48	41.63	1.69	0.059
Cross × Thermal history	5	1525.25	305.05	12.37	<0.001
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	0.002
history					
Residual	144	3550.99	24.66		
(b) Absolute growth rate, d8-d16					
Cross	5	19822.20	3964.44	23.00	<0.001
Temperature	3	145758.89	48586.30	281.90	<0.001
Thermal history	1	730.78	730.78	4.24	0.041
Cross × Temperature	15	14662.34	977.49	5.67	<0.001
Cross × Thermal history	5	11092.36	2218.47	12.87	<0.001
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					

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

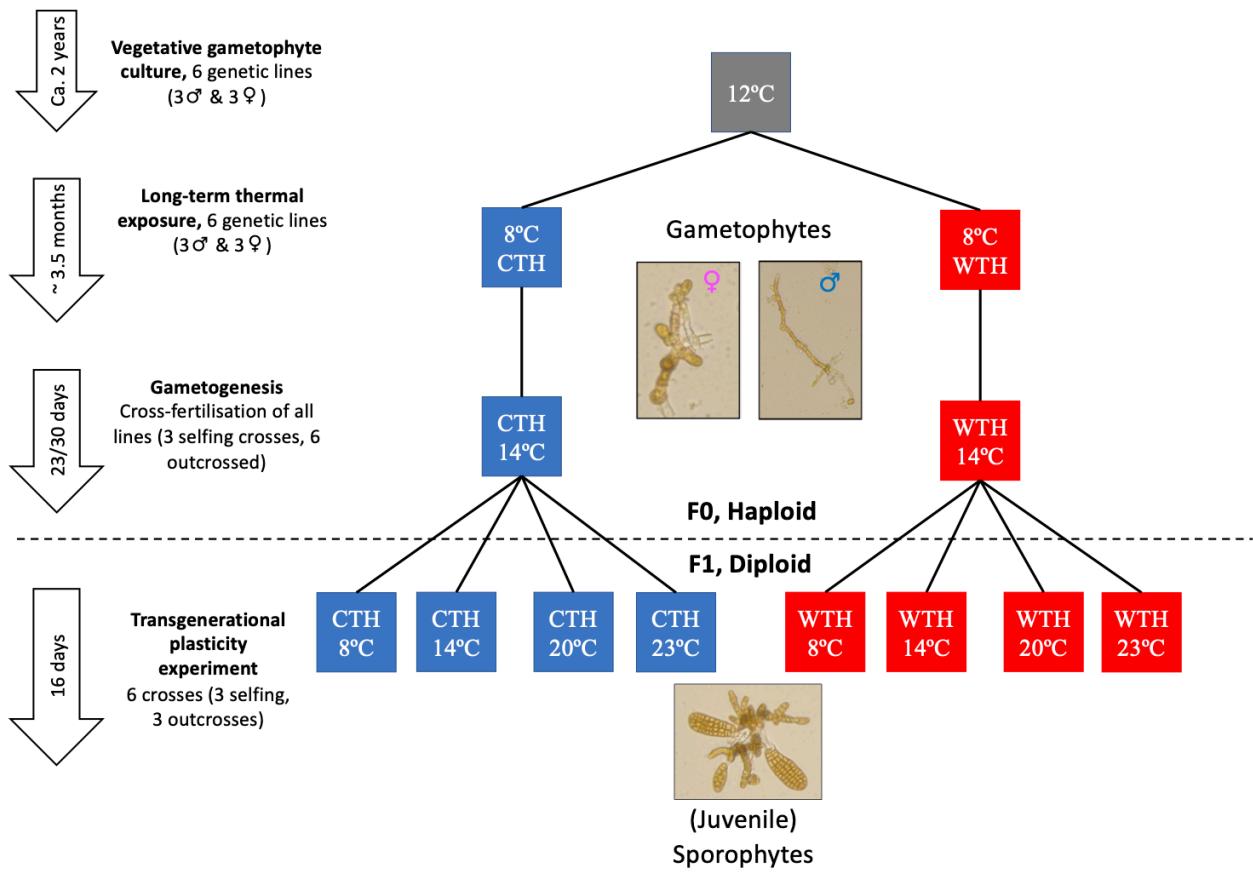
1 **Table S1.** Summary of the number of significant differences between thermal histories (8°C:
 2 CTH and 20°C: WTH) per experimental temperature (8, 14, 20 and 23°C) in the photosynthetic
 3 parameters of *Laminaria pallida* sporophytes.

Photosynthetic parameter	F_v/F_m	rETRmax		
Nº differences	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.

Time period	0-8 days		8-16 days	
Nº differences	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

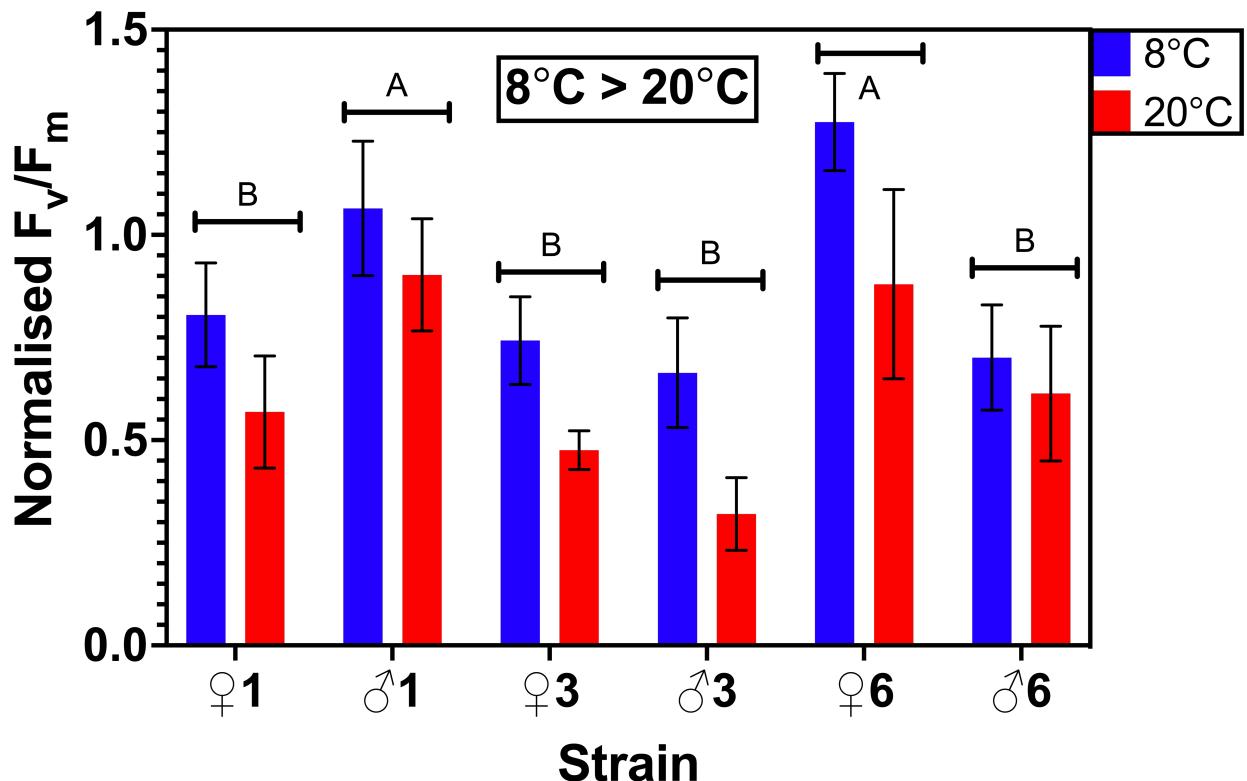
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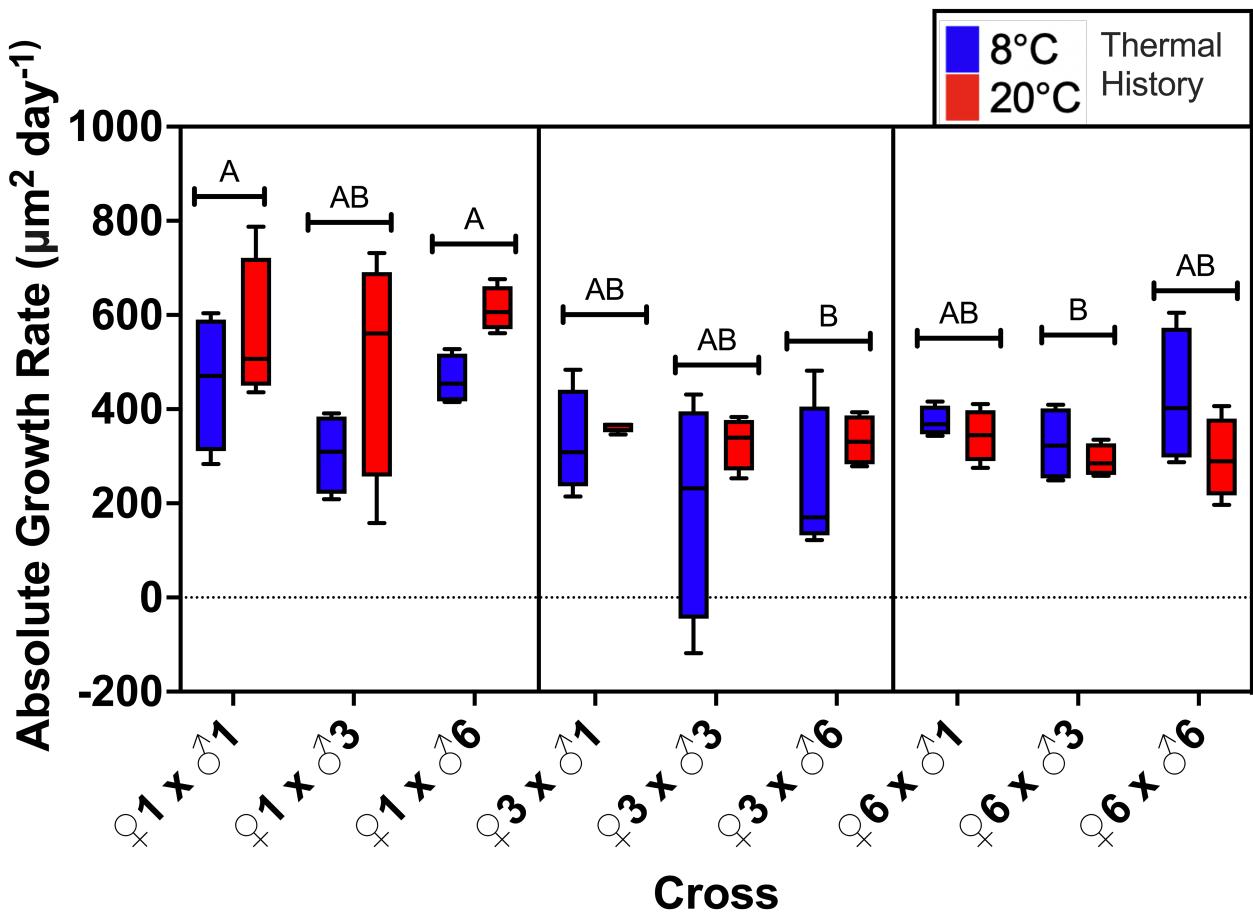
1

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).

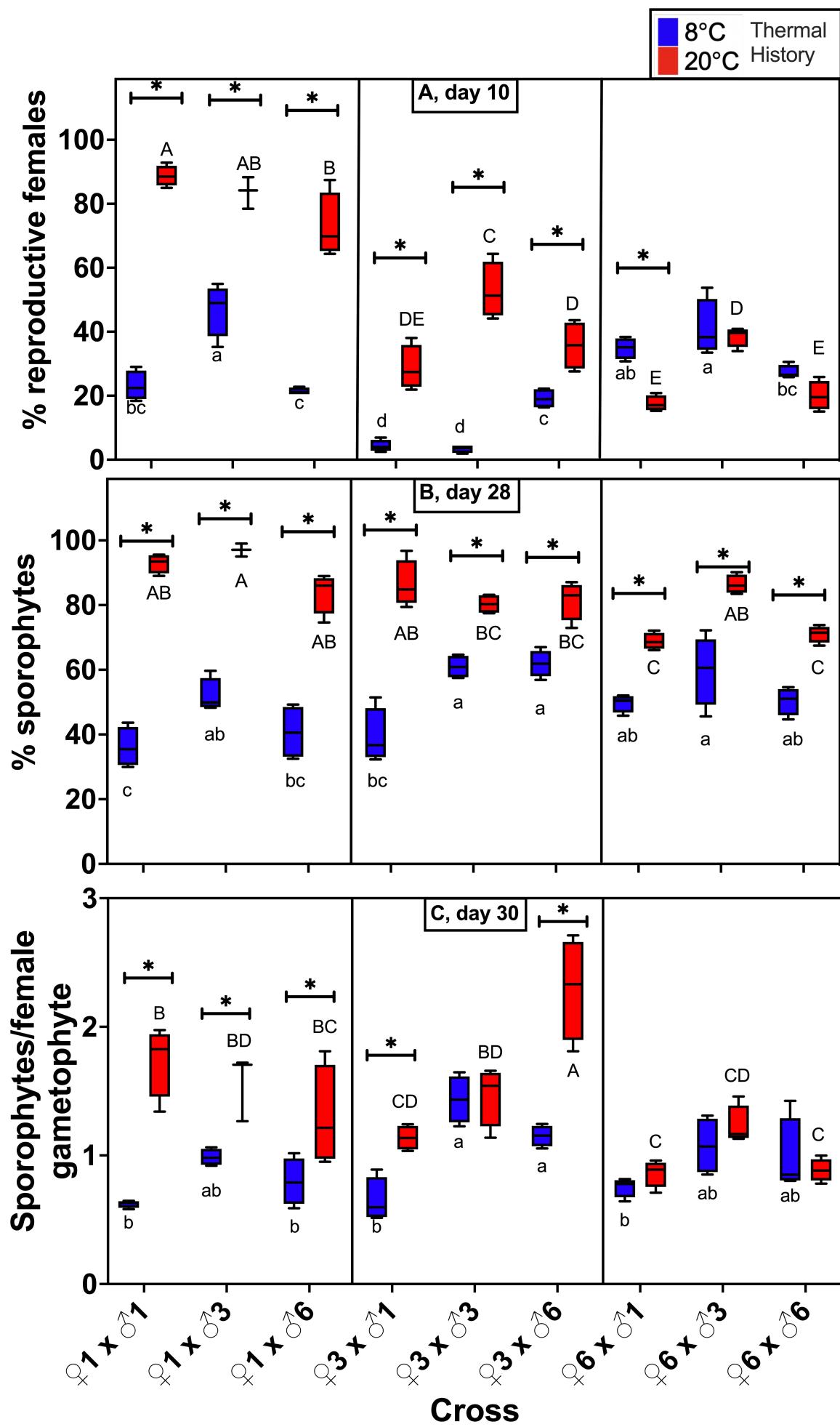
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1
2 **Figure 2: Effect of long-term temperature treatment (8°C and 20°C) on the maximum**
3 **photosynthetic yield of PSII (F_v/F_m) of different *Laminaria pallida* gametophyte strains.**
4 Please note that F_v/F_m values were normalized to the respective 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

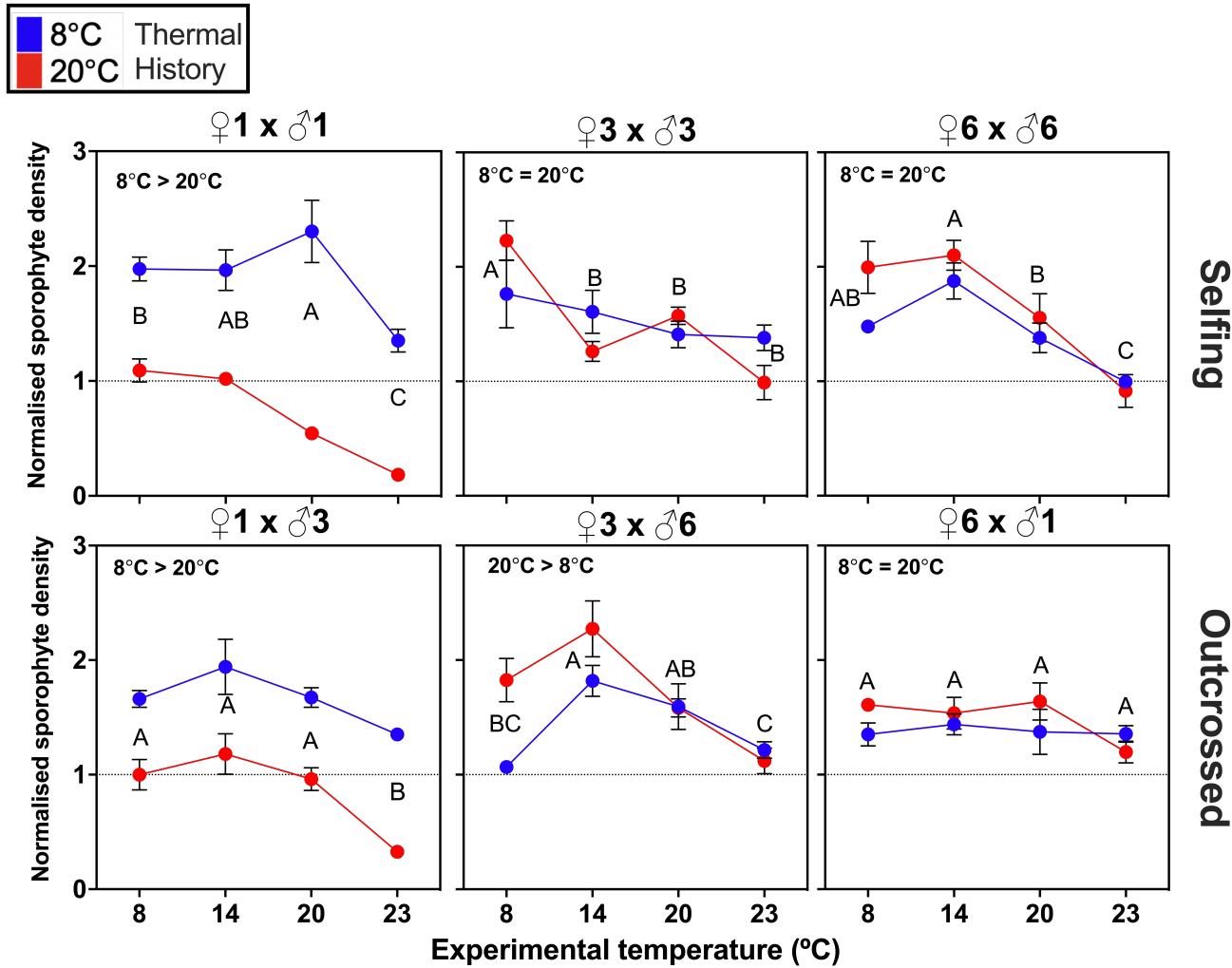


1
2 **Figure 3: Effect of thermal history on the absolute growth rate of gametophytes from**
3 **different *L. pallida* crosses after 6 days under gametogenic conditions.** Box plots with
4 median, boxes for 25th and 75th percentiles and whiskers indicating min and max values (n =
5 4). Panels separate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

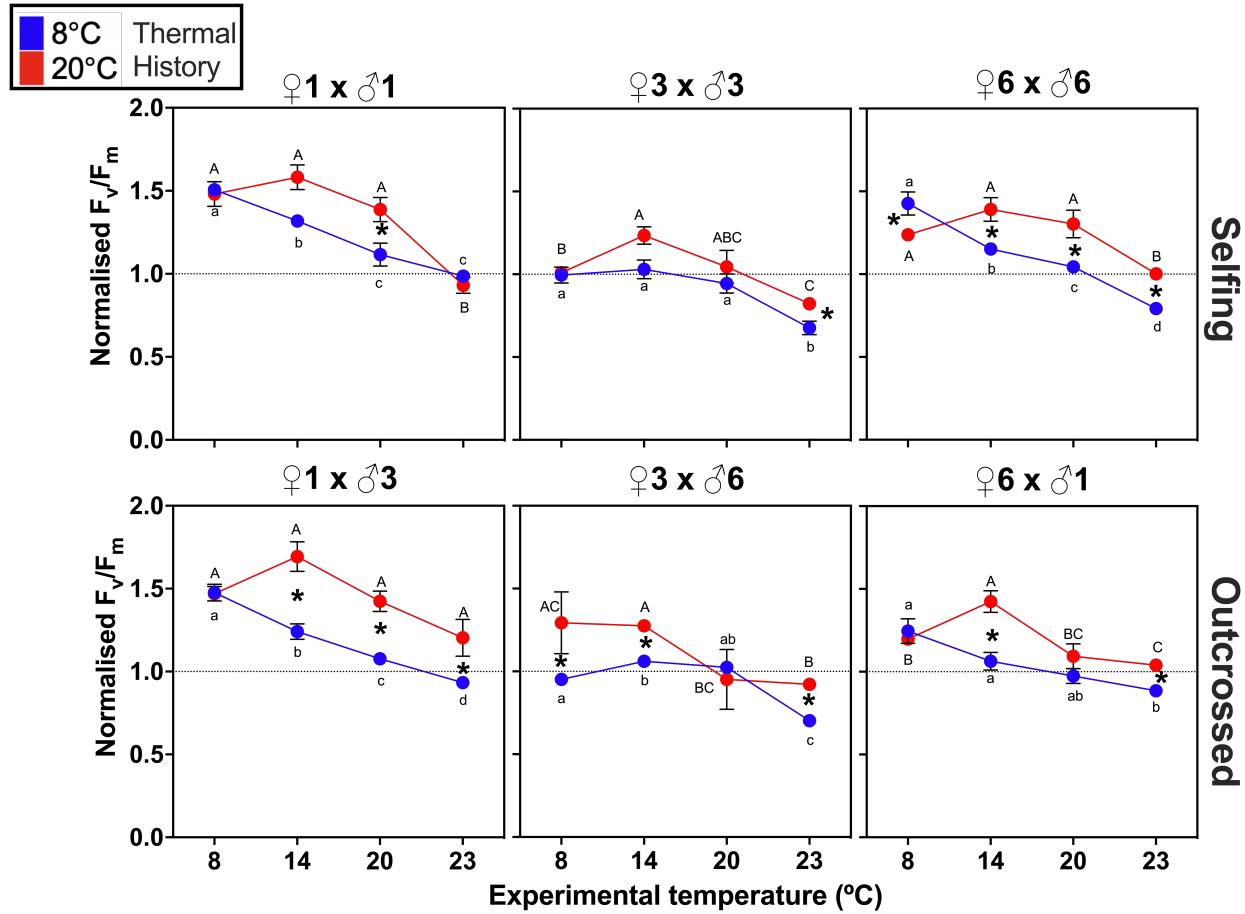


1 **Figure 4: Effect of thermal history on the reproductive success of different *L. pallida***
2 **crosses in gametogenic conditions.** (A) Percentage of reproductive female gametophytes (egg
3 released and sporophytes formed) after 10 days. (B) Percentage of female gametophytes with
4 sporophyte(s) after 28 days. (C) Absolute number of sporophytes per female gametophyte after
5 30 days. Box plots with median, boxes for 25th and 75th 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.

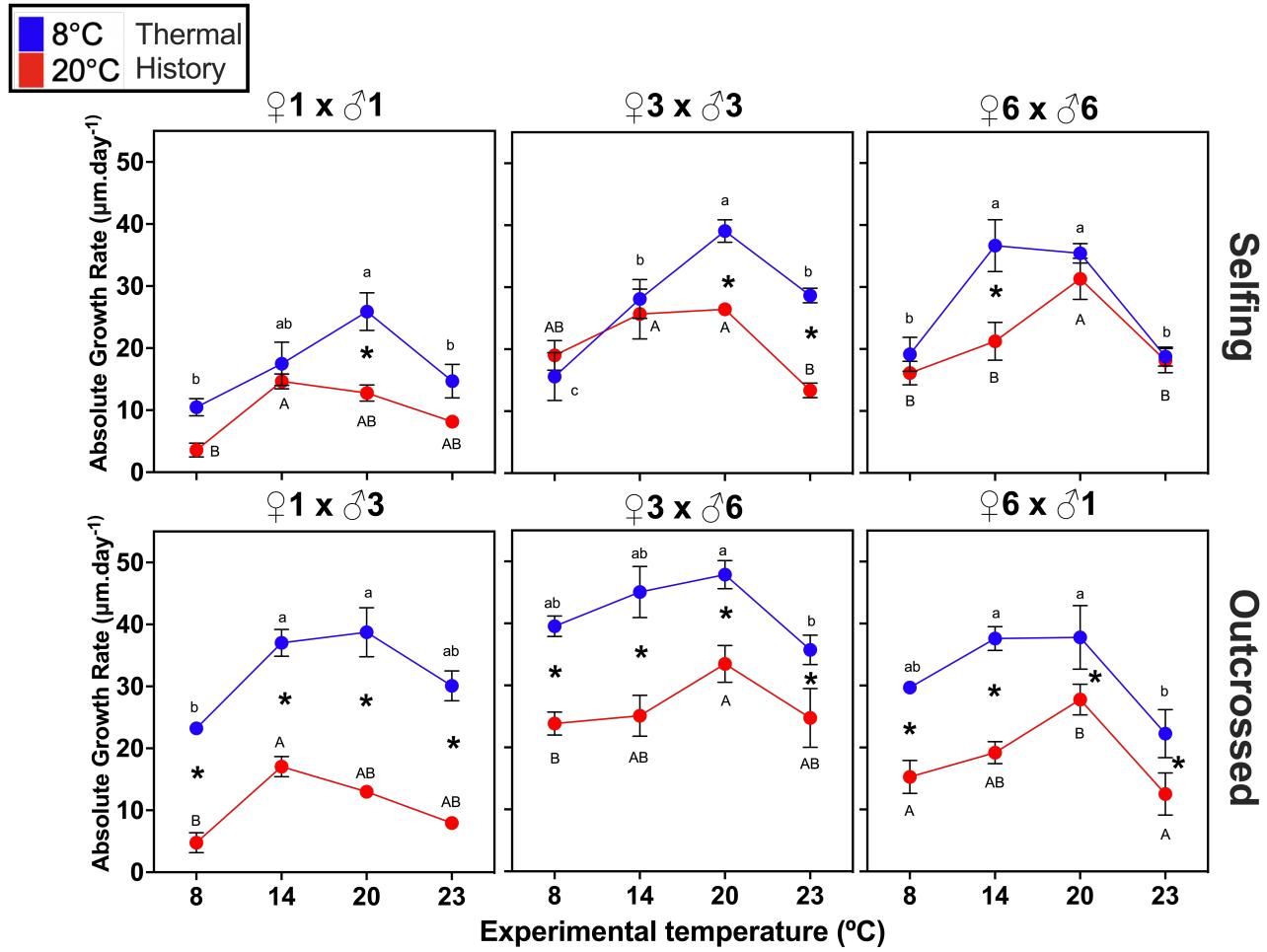
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1
2 **Figure 5: Effect of parental thermal history on the sporophyte density of different *L.*
3 *pallida* crosses after 16 days in experimental temperatures (8°C, 14°C, 20°C and 23°C).**
4 Connected mean plots with standard error of the mean (n = 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



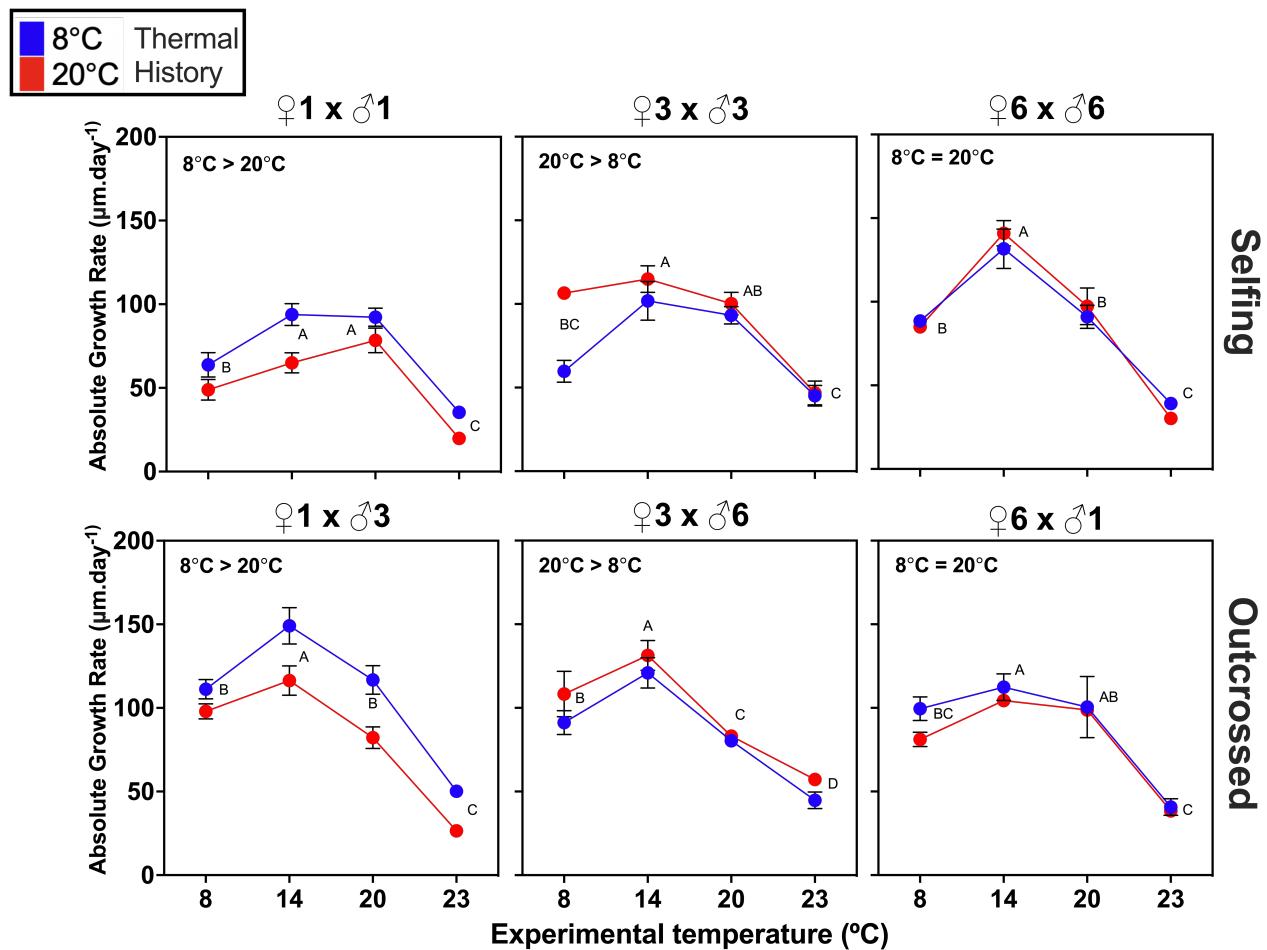
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2 **Figure 6: Effect on parental thermal history on the sporophyte maximum photosynthetic
3 yield (F_v/F_m) of different *L. pallida* crosses after 16 days in experimental temperatures
4 (8°C, 14°C, 20°C and 23°C). Connected mean plots with standard 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.

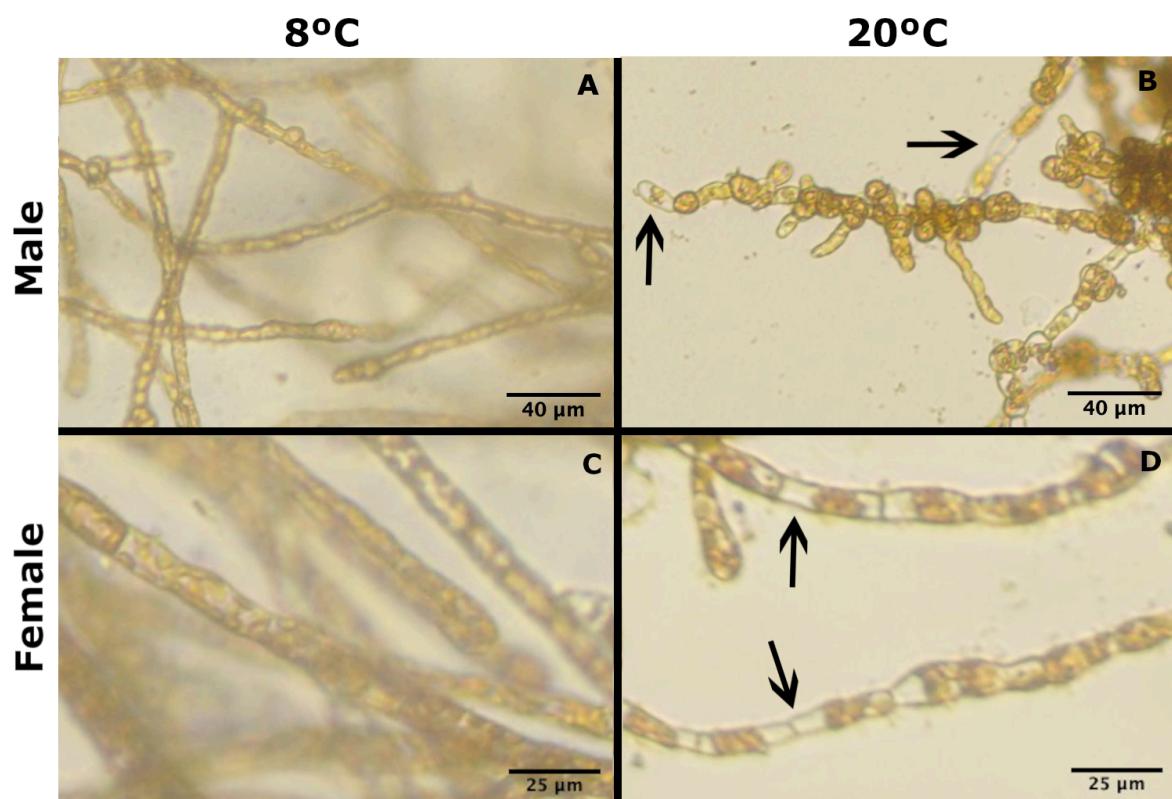
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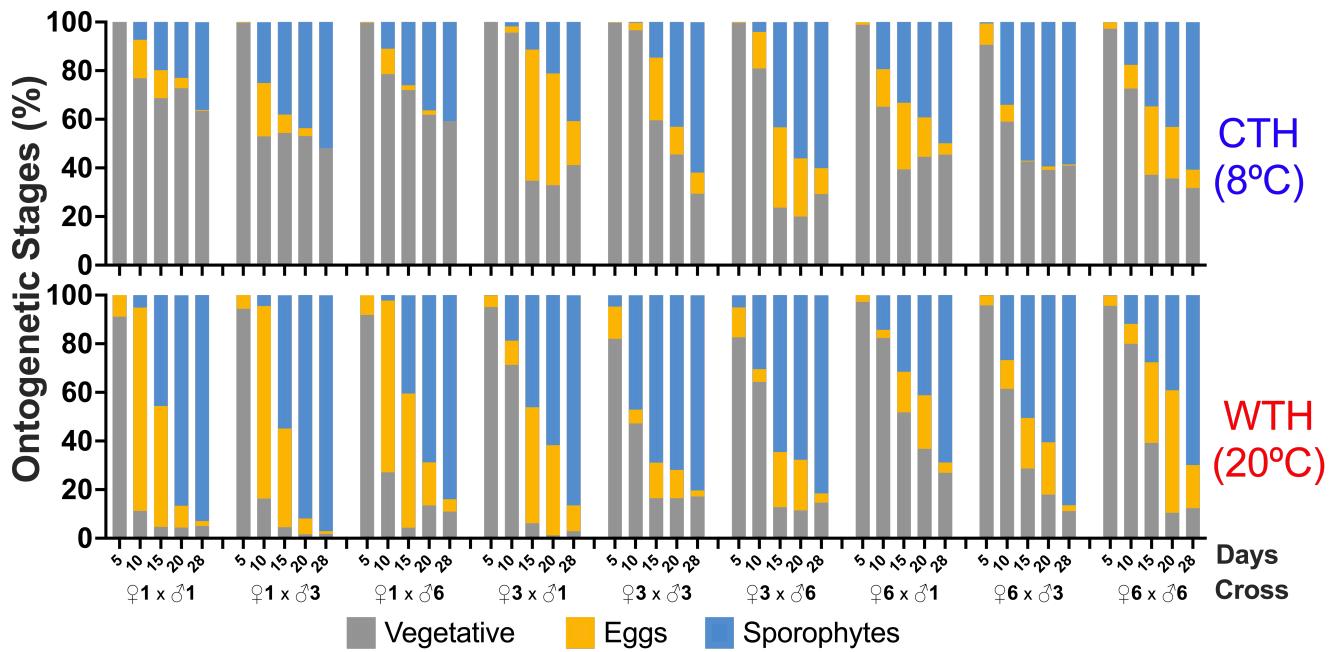
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2 **Figure 8: Effect of parental thermal history on the sporophyte absolute growth rate based**
 3 **on length of different *L. pallida* crosses between 8-16 days in experimental temperatures**
 4 **(8°C, 14°C, 20°C and 23°C).** Connected mean plots 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



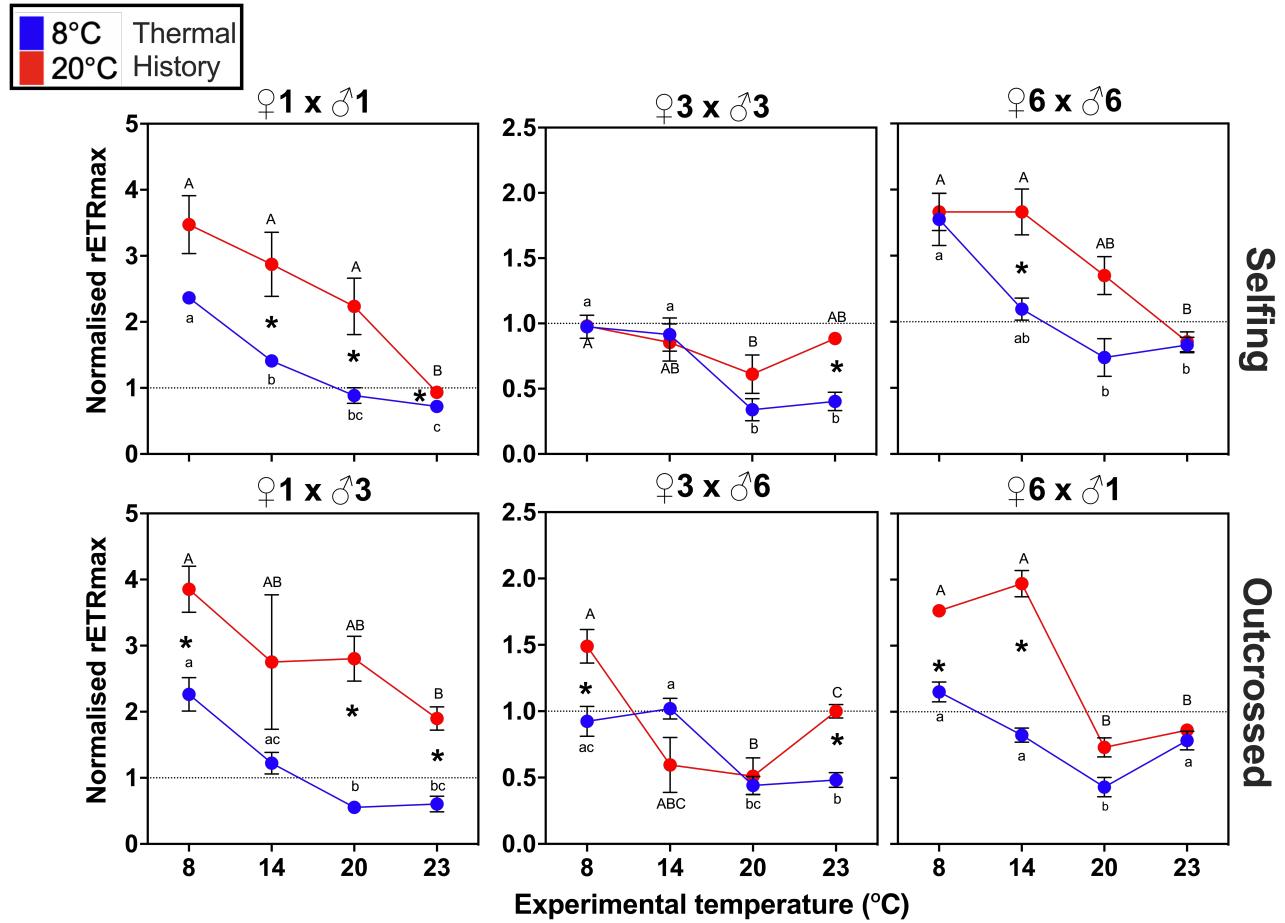
1
2 **Figure S1: Gametophytes of *Laminaria pallida* after ~3.5 months of temperature exposure**
3 **at 8°C and 20°C.** A, B. Male gametophyte cells. C, D. Female gametophyte cells. Arrows
4 indicate dead or damaged cells.
5



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