Effect of temperature variation in *Agarophyton chilensis*: contrasting the response of natural and farmed populations

Sara Usandizaga, Carolina Camus, José Luis Kappes, Marie-Laure Guillemin, Alejandro Buschmann

► To cite this version:

Sara Usandizaga, Carolina Camus, José Luis Kappes, Marie-Laure Guillemin, Alejandro Buschmann. Effect of temperature variation in *Agarophyton chilensis*: contrasting the response of natural and farmed populations. *Journal of Applied Phycology*, 2019, pp.1-9. 10.1007/s10811-019-1757-6 . hal-02148026

HAL Id: hal-02148026

<https://hal.sorbonne-universite.fr/hal-02148026>

Submitted on 5 Jun 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

[Click here to view linked References](#)

- 1 EFFECT OF TEMPERATURE VARIATION IN *AGAROPHYTON CHILENSIS*:
- 2 CONTRASTING THE RESPONSE OF NATURAL AND FARMED POPULATIONS
- 3 Sara Usandizaga
- 4 Programa de Doctorado en Ciencias, mención Conservación y Manejo de Recursos
- 5 Naturales, Centro i~mar and CeBiB, Universidad de Los Lagos, Camino Chiquihue Km 6,
- 6 Puerto Montt, Chile
- 7 Carolina Camus
- 8 Centro i~mar and CeBiB, Universidad de Los Lagos, Camino Chiquihue Km 6, Puerto
- 9 Montt, Chile
- 10 José Luis Kappes
- 11 Centro i~mar and CeBiB, Universidad de Los Lagos, Camino Chiquihue Km 6, Puerto
- 12 Montt, Chile
- 13 Marie-Laure Guillemin
- 14 Centro FONDAP de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes
- 15 (IDEAL), Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias,
- 16 Universidad Austral de Chile, Valdivia, Chile.
- 17 CNRS, Sorbonne Université, Pontificia Universidad Católica de Chile, Universidad Austral
- 18 de Chile, UMI Evolutionary Biology and Ecology of Algae, Station Biologique de Roscoff,
- 19 29680 Roscoff, France.
- 20 Alejandro H. Buschmann²

Centro i~mar and CeBiB, Universidad de Los Lagos, Camino Chiquihue Km 6, Puerto Montt, Chile

¹ Date of submission and acceptance

² Author for correspondence: Alejandro H. Buschmann, e-mail: abuschma@ulagos.cl, phone: +56 65 2322423

Running title: *Agarophyton chilensis* response to temperature variation

Abstract

During the domestication process, farmers influence the reproduction and care of organisms to ensure a predictable supply of the resource of interest, causing changes in phenotypic and genotypic character frequencies. In Chile, as a result of unconscious selection and domestication process, farmed populations of the red alga *Agarophyton chilensis* have most likely undergone a reduction in genetic diversity and a modification in life-history traits compared to wild populations. In order to understand the implications that these processes may have in *A. chilensis*, in this study we investigated how temperature variations (10 °C, 15 °C and 20 °C) affect growth and photosynthetic responses of natural and farmed populations from three different localities along the Chilean coast. Natural population's growth decreased at low and high temperature levels while all three farmed populations respond in a very similar way to temperature variation. We propose that a possible outcome of farming, in the *A. chilensis* vegetatively propagated crops, could have been the selection of general-purpose-genotypes able to perform adequately across the range of temperature tested in our experiment. Furthermore, our results showed that photosynthetic activity was also affected by temperature treatments (e.g., different maximum maximal electron

transport rate and quantum yield values depending on the population type and temperature). In a context of climate change, *A. chilensis* farmed populations may be better able to cope with impacts of anthropogenic activities than natural populations due to the buffer effect of their general-purpose-genotypes, tolerant to a wide range of conditions.

Key index words: abiotic factor, domestication, management, general-purpose-genotypes, photosynthesis, origin, seaweed, selection.

Abbreviations: ETR_{max} , maximal electron transport rate; F_v/F_m , maximal quantum yield; E_k , irradiance of saturation of ETR; NPQ_{max} , maximal non-photochemical quenching; PAR, photosynthetically active radiation; PSII, photosystem II; RLC, rapid light curves.

Introduction

Domestication is considered a long and complex process during which domesticators influence the reproduction and care of domesticated species to guarantee predictable supply of resources presenting selected traits of interest for human use (Zeder, 2015). This selection process generates changes in phenotypic and genotypic character frequencies of cultivated populations (Zohary, 1984). Even if domestication of marine species is much more recent than the one of terrestrial animals and plants (Duarte et al. 2007), strong evidence for domestication has been found in a few cultivated seaweeds (Valero et al. 2017). As for terrestrial plants (Meyer et al. 2012), some domesticated seaweeds are characterized by a shift in their reproductive strategy (e.g., changes from sexual reproduction to vegetative propagation) between natural and farmed populations (Valero et al. 2017). This shift in reproductive strategy has been demonstrated for *Agarophyton* (referred as *Gracilaria* in Guillemain et al. 2008) and is probably also present in

65 *Kappaphycus* (Ask and Azanza 2002). Asexual propagation enables farmers to selectively
66 multiply superior genotypes and maintain desired phenotypes through vegetative
67 propagation (Valero et al. 2017).

68 In Chile, intensive seaweed farming is limited to the domesticated red alga *Agarophyton*
69 *chilensis* used mainly for agar extraction (Buschmann et al. 2017). Even if the
70 domestication process of this species has begun only a few decades ago, the almost
71 complete predominance of diploid individuals in farms demonstrate that farming practices
72 had significantly modified life-history traits as compared to wild populations (Guillemin et
73 al. 2008). Moreover, recent investigations have demonstrated that this red alga colonized
74 the Chilean coast from New Zealand, likely at the end of the Last Glacial Maximum
75 (Guillemin et al. 2014). The lower genetic diversity of the Chilean populations, when
76 compared to the ones from New Zealand, is indeed consistent with a genetic bottleneck
77 resulting from a transpacific range extension and was probably reinforced by the over-
78 exploitation of natural Chilean populations during the 90's (Guillemin et al. 2014). In
79 Chile, active transport and exchange of inoculums of *A. chilensis* between coastal
80 communities of fishermen for cultivation purposes have contributed to the artificial
81 expansion of the species distribution. Nowadays, natural populations are distributed
82 between 30°S to 45°S while farming extends further north, up to Antofagasta, 17°S (Bird et
83 al. 1986, Guillemin et al. 2008). Human activities have also led to a loss of genotypic
84 diversity in farmed populations that could be partially linked to involuntary selection of
85 faster growing thalli, during the first steps of the domestication process (Guillemin et al.
86 2008, Guillemin et al. 2013, Valero et al. 2017).

87 Reduced genetic diversity can severely affect the ability of populations to resist pests and
88 pathogens and limit the scope for future genetic improvement in domesticated crops
89 (Robinson et al. 2013). Besides, it has been reported that a reduction in genetic diversity
90 could lead to lower growth and resilience in highly stressful and/or variable environment
91 (Simms 2000). However, to date, few studies have focused on the potential
92 ecophysiological differences between natural and farmed populations of *A. chilensis*.
93 Gallegos-Sánchez et al. (2018), detected a significant and negative effect of low salinity
94 conditions on thalli sampled from both natural and farmed *A. chilensis* populations but with
95 farmed population's thalli being less affected than the natural ones. Results suggested that
96 farmed populations might be more tolerant to salt stress than wild ones in this species and
97 the authors proposed that this difference between population types could be due to previous
98 selective process carried out by farmers.

99 Considering the possible consequences of unconscious selection (as defined in Zohary
100 2004) and domestication on *Agarophyton chilensis*, we propose that natural populations
101 will be less sensitive to temperature variations than farmed populations. Genotypic
102 diversity has been shown to be higher in natural than farmed *A. chilensis* Chilean
103 populations and we propose that the farms will show less effective mechanisms of
104 acclimation (e.g., enhancing of photosynthetic performance) than the one observed in
105 natural populations when confronted with temperature considered as high in their natural
106 environment (i.e., water of the southern coast of Chile do not generally reach 20°C;
107 Westermeier et al. 1993). The aim of the present study was to assess the effect of
108 temperature on growth and photosynthetic responses of both natural and farmed stands of
109 *A. chilensis* and tetrasporophyte thalli sampled from three sites along the Chilean coast

were followed during one month in controlled laboratory conditions at 10°C, 15°C and 20°C.

Materials and methods

Study sites and life cycle phase determination of the sampled thalli

A total of 600 individuals were sampled from 3 natural and 3 farmed populations (i.e., 100 individuals in each population) during a spring season. One farmed and one natural population were sampled from three sites: Concepción, Maullín and Ancud (Fig.1). The distinction between natural and farmed populations was first based on whether *Agarophyton chilensis* thalli were actively planted or not by farmers. As previously reported for the species (Guillemin et al. 2008), thalli were attached to small rocks and pebbles by a holdfast in the three natural *A. chilensis* beds while unattached thalli were found embedded in the sandy bottom in the three farmed stands. In each site, natural and farmed populations were separated by 1 km approximately. In natural beds, individuals sampled correspond to distinct holdfasts (i.e., distinct genotypes produced by sexual reproduction and spore settlement). In farms, to avoid sampling fragments of the same asexually propagated genotype, sampled thalli were separated by at least by 2 m. All the collected thalli were transported in isolated boxes to the CEACIMA hatchery (Centro de Investigación de Acuicultura y Ciencias del Mar, Universidad de Los Lagos) located in the Metri Bay (41°36'S, 72°43'W). Once in the hatchery, each thallus was cleaned with fresh water and all epiphytes were removed by hand. Thalli were individually marked with a numbered tag and maintained in 400 L tanks at 12°C with constant aeration, 12L:12D photoperiod, 20 $\mu\text{mol electron m}^{-2} \text{s}^{-1}$ photon flux and weekly filtered seawater exchange.

Collected thalli were observed under stereoscope microscope (Stemi DV4, Zeiss, Jena, Germany) to determine phase (i.e., diploid tetrasporophytes or haploid gametophytes) of mature individuals. For vegetative individuals, a 3-cm fragment of tissue was excised from each thallus, placed into plastic bags with silica gel for rapid dehydration. The sex markers available for *A. chilensis* were amplified following Guillemín et al. (2012) and the amplification products were visualized in 1.5 % agarose gel (w/v) after adding 2 µl of GelRed™ (Biotium, Fremont, USA). Results were used to determine sex and phase of vegetative individuals. In order to prevent experimental bias due to ecophysiological variability between life cycle phases (Guillemín et al. 2013), only diploid tetrasporophytes were selected for our experiments.

Experimental design

The experimental design consisted of 90 2-L Erlenmeyer flasks (i.e., 6 populations of origin of thalli x 3 temperature treatments x 5 replicates per population per temperature treatment) arranged in 15 60-L plastic water tanks fitted with temperature control systems. Three temperature treatments were used: 10°C, 15°C and 20°C. These temperatures were chosen since they roughly represent the temperature range encountered in the field by the study species. Temperature conditions varied widely within sites where *A. chilensis* is found and between regions populated by the species along the Chilean coasts with values between 9 °C and 16 °C recorded in Maullín (Westermeyer et al. 1993) and between 10 °C and 20°C in areas further north such as Concepción, Coquimbo and Antofagasta (Santelices and Ugarte 1990). In each 60-L plastic water tanks, one 50W automatic heater (Whale VK-1000, Regent) and one stainless steel thermometer (Hagen, Phelan) were used to maintain constant temperature. For each population under study, five replicates (i.e., five 2-L flasks)

were followed per temperature treatment and eight thalli (5 cm length each), selected randomly from distinct tetrasporophytes, were placed in each 2-L flask. Thalli were selected without replacement from a pool of 40 tetrasporophytes, available for each population of origin (see above). Once a week, thalli were transferred to clean 2-L flasks with fresh Provasoli culture media (McLachlan 1973). After one week of acclimation at 12°C, the laboratory experiment was run during 30 days. All Erlenmeyer flasks were under constant conditions of aeration, photoperiod (12 h light: 12 h dark) and photon flux (20 $\mu\text{mol electron m}^{-2} \text{ s}^{-1}$).

Growth

Fresh weight of each thallus was assessed weekly on an analytical balance (Sartorius TE 313 DS, Germany) and the specific growth rate (SGR) was calculated as the percentage of wet weight gain per day according to the formula: $\text{SGR} = [\ln (W_f \cdot W_i^{-1}) / (t_f - t_i)] \times 100$; where W_i = initial fresh weight, W_f = final fresh weight, and t = time (days). Initial fresh weight in the 2-L flasks was of 0.12 ± 0.06 g, some variation in fresh weight exist between each 2-L flask at the beginning of the experiment since calibration of algal material was based on thallus length (see above).

Physiological variables

Thallus pieces were collected at the end of the experiment to measure rapid light curves (RLC). As an indicator of quantum efficiency and photoinhibition, we used, F_v/F_m , which was determined after incubation of 20 min of the thalli in darkness (Schreiber et al. 1995) with a Junior PAM (Walz GmbH, Effeltrich, Germany). The electron transport rate (ETR, $\mu\text{mol electrons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) was determined after 20 s exposure in 12 increasing intensities of

PAR (up to 1500 $\mu\text{mol photon} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) provided by a blue light of the Junior PAM device (Schreiber et al. 1995). ETR was calculated according to Schreiber et al. (1995) as follows:

$$\text{ETR} = \Delta F/F'_m \cdot E \cdot A \cdot F_{\text{II}} \text{ (}\mu\text{mol electrons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\text{)} \quad (1)$$

Where $\Delta F/F'_m$ is the effective quantum yield, E is the incident PAR (photosynthetically active radiation) irradiance expressed in $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, A is the thallus absorptance and F_{II} is the fraction of chlorophyll related to photosystem II (PSII, 400-700nm), being 0.15 in red seaweed (Grzyski et al. 1997). As an estimator of photosynthetic efficiency, the initial slope of ETR (α_{ETR}) and maximum ETR (ETR_{max}) were obtained from the tangential function reported by Eilers and Peeters (1988) and the irradiance saturation ($E_{k_{\text{ETR}}}$) was calculated from the intercept between these two parameters. Representing potential thalli photoprotective mechanism, non-photochemical quenching (NPQ) was measured according to Schreiber et al. (1995). The maximal non-photochemical quenching (NPQ_{max}) was calculated from the tangential function of NPQ versus irradiance function according to Eilers and Peeters (1988).

Statistical treatment

All analyses were performed in R (3.2.4 version) (Cayuela 2011). Assumptions of homogeneity of variances and normal distribution were tested using Levene's test and Shapiro-Wilk, respectively. When non-normal residuals and heteroscedasticity were detected, the data were transformed using logarithm (for E_k and SGR) or Box-cox (for F_v/F_m). The experimental design fitted a three-way ANOVA with treatment (temperature: 10°C, 15°C and 20°C), site of origin (Concepción, Maullín and Ancud) and population type

(natural or farmed) considered as fixed factors. Statistical differences between groups were analyzed using comparisons of means (Tukey's HSD). Significances were set at $p < 0.05$.

Results

Significant differences were detected in *Agarophyton chilensis* specific growth rate (SGR) after 30 days of experimentation between temperature treatments ($F_{(2, 72)} = 15.46$; $P < 0.0001$), between population types ($F_{(1, 72)} = 4.93$; $P = 0.03$) and a significant interaction was also detected between population type and site of origin ($F_{(2, 72)} = 5.00$; $P = 0.009$). In Maullín and Ancud, no significant differences between temperature treatments were detected for farmed thalli (Fig. 2C and E), while farmed thalli from Concepción showed a slightly but significantly lower SGR at 20°C than at 15°C (7.26 ± 1.48 and 9.45 ± 1.92 g · day⁻¹, respectively; Fig. 2A). In addition, differences among Concepción farmed and natural populations were observed (Tukey test; $P < 0.05$, see Figure 2A and B). In contrast, SGR was significantly higher at 15°C for thalli sampled in natural *A. chilensis* stands, whatever the site under study (SGR at 15°C: 7.49 ± 2.07 g · day⁻¹ in Concepción, 8.81 ± 2.60 g · day⁻¹ in Maullín and 8.67 ± 0.84 g · day⁻¹ in Ancud; Fig. 2B, D and F). Finally, the growth rate did not decrease significantly (Tukey test; $P < 0.05$) at the lower (10 °C) and the higher (20 °C) temperature tested for Maullín and Ancud populations (Fig. 2 C, D, E and F).

Regarding the photosynthetic responses, significant effect of temperature treatments were detected for all parameters measured: the optimal quantum yield of fluorescence (F_v/F_m), the maximal electron transport rate (ETR_{max}), the saturation irradiance (E_k) and the maximal non-photochemical quenching (NPQ_{max}) (see Table 1). Interactions between temperature treatment and population type were also observed for ETR_{max} and E_k (Table 1). Values of

ETR_{max} and *Ek* were significantly higher for thalli sampled in the farmed population of Concepción and grown at 20°C than for thalli sampled in the farmed population of Ancud and grown at 10°C (18.63 ± 12.76 and 1.44 ± 0.37 $\mu\text{mol electrons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 442.97 ± 306.60 and 31.86 ± 4.78 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, for ETR_{max} and *Ek*, respectively; Tukey's HSD tests; $p < 0.05$; Table 2). Significant interactions between temperature treatment, population type and site of origin were observed for F_v/F_m and NPQ_{max} (Table 1). In Maullín at 15°C, values of NPQ_{max} were significantly higher for thalli sampled in the farmed bed than the natural population (Tukey's HSD tests; $p < 0.05$; Table 2).

Discussion

The present study confirms that natural and farmed *Agarophyton chilensis* populations respond differentially to temperature variations. Indeed, contrarily to our expectations, our results indicate that farmed populations are less sensitive to temperature variations than natural ones (i.e., thalli growth rate are mostly similar at 10°C, 15°C and 20°C for farms while thalli from natural populations have a higher growth rate at 15°C). In Chile, farmed population has been under unconscious human selection pressure for, at least, three decades and we propose that a possible outcome could have been the selection of general-purpose-genotypes (as in Baker 1974) in these vegetatively propagated crops. Our results also showed that photosynthetic activity was affected by temperature treatments (e.g., different maximum maximal electron transport rate and quantum yield values depending on the population type and temperature).

Regardless of the origin of the natural population under study, our experiment shows that thalli specific growth rate (SGR) was higher at 15°C than at lower (i.e., 10°C) or higher (i.e., 20°C) temperatures. Even if SGR tended to be much more homogeneous for farmed

thalli at all temperatures, one slight difference was observed for the farmed thalli from Concepción that show significantly less growth at 20°C. Supporting our results, a study realized by Santelices and Ugarte (1990) on *A. chilensis* (as *Gracilaria chilensis*) natural populations from Maullín, also revealed better growth rates at 15°C than at 10°C or 20°C. Most temperate species of the genus *Agarophyton* has been shown to grow faster in temperature ranging between 15° and 20°C (McLachlan and Bird 1984). A study performed in *Gracilaria gracilis* (as *G. verrucosa*) reported a slow growth rate at 10°C and high mortality after a 14 days' heat-wave in Saldanha Bay, South Africa (Engledow and Bolton 1992). *A. chilensis*, is a temperate-water species with a distribution limited to southern part of the Pacific (Bird et al. 1986, Guillemin et al. 2008) and it is possible that the species present a metabolism with limited temperature tolerance.

Although farming has begun only a few decades ago (i.e., during the 80's) in Chile, the predominant mechanism for stock propagation by cuttings used in *Agarophyton chilensis* farms has already significantly decreased their genotypic diversity when compared with wild populations (Guillemin et al. 2008). There is an overall agreement that genetically more variable populations may be associated with higher resilience, increased productivity and population growth rate as compared with less variable populations (Forsman 2014). However, contrasting with our expectations, farmed thalli in our experiment clearly show a strong ability to grow in contrasting environmental conditions, including the quite “extreme” temperature of 20°C tested. In the same way, Gallegos-Sanchez et al. (2018) concluded that *A. chilensis* farmed populations may be less sensitive to salt stress and able to grow in a greater range of salinity than natural populations. One possible explanation for these results is that farmed populations of *A. chilensis* are composed mostly of general-

purpose genotypes, able to grow in highly stressful and/or variable environments. General-purpose-genotypes are sometime also referred as ‘Jack-of-all-trades, master of none’ since they are described as versatile genotypes that are able to perform adequately across a range of environments but are not superior in any of them. These general-purpose-genotypes can confer a species or population a broad tolerance to environmental changes and are often associated with species invasion (Baker 1974, Richards et al. 2006). In Chile, farms have been developed using material growing embedded in muddy estuaries and sandy bays. These habitats are typically highly heterogeneous and present strong seasonal variations in temperature and salinity (Westermeyer et al. 1993, Buschmann et al. 1995). It have been demonstrated that intraspecific competition for resources utilization in clonal individuals living in habitats characterized by fluctuating environmental conditions could lead to positive selection of general-purpose-genotypes (Arnaud-Haond et al. 2012). In benthic algae, temperature variations affect photosynthetic metabolism (Davison 1991) determining, for example, seasonal distribution (De Nicola 1996). However, habitats characterized by strong spatial and temporal variations of abiotic factors, request constant adjustment of photosynthetic processes in species populating them (Ensminger et al. 2001). In our study, the highest values of ETR_{max} and E_k were observed in the *A. chilensis* farmed population from Concepción grown at 20°C. These results suggest that the effect of high temperatures on photosynthetic metabolism of this farmed population could be mild. Driven by seasonal changes in river discharge, precipitation, and coastal upwelling, high variability in abiotic conditions (e.g., temperature, salinity and turbidity; Saldías et al. 2016) has been observed in the river mouths where *A. chilensis* thalli are planted in Concepción. These characteristics could be associated to distinctive heat susceptibility of the photosynthetic metabolism of the *A. chilensis* thalli growing in Concepción farms. In

our experiment, the response of the maximum quantum yield (F_v/F_m) to temperature was quite variable. The lowest value of F_v/F_m (indicating photoinhibition) was observed at intermediate temperature (15°C) in the farm of Maullín. In plants, it is well known that limitation of electron transport that reduces the ability of plants to use light result in an excess light energy that may cause photoinhibition due to damage to the PSII apparatus (Moll and Steinback 1986). However, short-term response of photosynthesis to temperature cannot easily be used to infer the long-term response of algal growth (Wienke and Dieck 1989). Indeed, despite the possible signal of photoinhibition detected at 15°C, no limited growth could be observed in these *Agarophyton chilensis* thalli.

Populations of *Agarophyton chilensis* from different sites in Chile have been reported to present ecological differences, potentially linked to local adaptation in response to specific abiotic and biotic environmental characteristics (Santelices and Ugarte 1990, Usandizaga et al. 2018). The success of *Agarophyton* farming depends in part on the origin of the initial inoculum since differences in thallus morphology, agar yield and gel strength and susceptibility to epiphytes exist among regions and populations. Indeed, it have been suggested that random transplantation between distinct habitats could lead to cultivation failure (Santelices and Ugarte 1990). However, in our experiment, even if we included sampling sites located more than 700 km apart, no major effect of the site of origin were detected on growth or photosynthesis. A possible explanation for this discrepancy is that continuous transplantations and exchanges during the last decades have leaded to the homogenization of the genetic diversity among the whole Chilean coast. However, this hypothesis is not in accordance with population genetic studies showing the presence of clear genetic divergence between regions in Chile (Guillemin et al. 2008 and 2014). Studies focused on the effect of other stressors (e.g., nutrient supply, salinity, irradiance) and the

cumulative effects of various of these stressors on the physiological responses of distinct genotypes is now needed in order to better explore the resistance of *A. chilensis* populations to stress.

Conclusion

Agarophyton chilensis tolerance of a wide range of abiotic conditions has been proposed as one of the main reasons of the species successful expansion in the Pacific and establishment in a wide array of habitats (Santelices and Ugarte 1990, Chow et al. 2001). These successful extension waves have probably also been facilitated by intrinsic characteristics of the species, such as its capacity to shift between sexual and asexual reproduction (Guillemin et al. 2014). We suggest that the possible selection for general-purpose-genotypes in the asexually reproducing farmed populations may help modulating the impact of environmental variation on population dynamics (Reed et al. 2010) and Chilean *Agarophyton* crop to better cope with impacts of climate change and direct anthropogenic activities. Nevertheless, implementation of breeding strategy and cultivar selection for mariculture systems improvement has not yet begun in Chile and development of long-term management plans for the sustainable exploitation of *A. chilensis* populations is dearly needed.

Acknowledgements

This research was supported by a doctoral fellow from Universidad de Los Lagos (Chile) to SU, CeBiB (CONICYT, FB-0001) to AHB and CC and Fondecyt N°1150987 and N°1170541 (CONICYT) to AHB and MLG, respectively. Additional support was provided by the grant “Término de Tesis” awarded by Universidad de Los Lagos. The

authors are grateful for the help in the field of R. Altamirano and S. V. Pereda for the revision of this manuscript. We also wish to thank two anonymous referees who greatly improved the manuscript with constructive suggestions. The authors declare no conflict of interest.

References

- Arnaud-Haond S, Duarte C, Diaz-Almela E, Marbà N, Sintes T, Serrão (2012) Implications of extreme life span in clonal organisms: millenary clones in meadows of the threatened seagrass *Posidonia oceanica*. PloS one 7:e30454.
- Ask EI, Azanza RV (2002) Advances in cultivation technology of commercial eucheumatoid species: a review with suggestions for future research. Aquaculture 206:257-277.
- Baker HG (1974) The evolution of weeds. Annu Rev Ecol Syst 5:1-24.
- Bird CJ, McLachan J, Oliveira EC (1986) *Gracilaria chilensis* sp. nov.; (Rhodophyta, Gigartinales), from Pacific South America. Can J Bot 64:2928-2934.
- Buschmann AH, Westermeier R, Retamales C (1995) Cultivation of *Gracilaria* in the sea-bottom in southern Chile: a review. J Appl Phycol 7:291–301.
- Buschmann AH, Camus C, Infante J, Neori A, Israel A, Hernández-González MC, Pereda SV, Gomez-Pinchetti JL, Golberg A, Tadmor-Shalex N, Critchley AT (2017) Seaweed production: overview of the global state of exploitation, farming and emerging research activity. Eur J Phycol 52:391–406.
- Cayuela L (2011) Modelos lineales: Regresión, ANOVA y ANCOVA. Universidad Rey Juan Carlos, Madrid.

360 Chow FY, Macchiavello J, Cruz SS, Fonck E, Olivares J (2001) Utilization of *Gracilaria*
 361 *chilensis* (Rhodophyta: Gracilariaceae) as a biofilter in the depuration of effluents from
 362 tank cultures of fish, oysters, and sea urchins. J World Aquac Soc 32:215-220.

363 Davison IR (1991) Environmental effects on algal photosynthesis: temperature. J Phycol
 364 27:2–8.

365 De Nicola DM (1996) Periphyton responses to temperature at different ecological levels.
 366 In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology: freshwater benthic
 367 ecosystems. Academic Press, San Diego, pp 149-181.

368 Duarte CM, Marbá N, Holmer M (2007) Rapid domestication of marine species. Science
 369 316:382.

370 Eilers PHC, Peeters JCH (1988) A model for the relationship between light intensity and
 371 the rate of photosynthesis in phytoplankton. Ecol Model 42:199-215.

372 Engledow HR, Bolton JJ (1992) Environmental tolerances in culture and agar content of
 373 *Gracilaria verrucosa* (Hudson) Papenfuss (Rhodophyta, Gigartinales) from Saldanha Bay.
 374 S Afr J Bot 58:263-267.

375 Ensminger I, Xyländer M, Hagen C, Braune W (2001) Strategies providing success in a
 376 variable habitat: III. Dynamic control of photosynthesis in *Cladophora glomerata*. Plant
 377 Cell Environ 24:769-779.

378 Forsman A (2014) Rethinking phenotypic plasticity and its consequences for individuals,
 379 populations and species. Heredity 115:1–9.

380 Gallegos-Sánchez CF, Beltrán J, Flores V, González AV, Santelices B (2018) Testing the
 381 effects of heterozygosity on growth rate plasticity in the seaweed *Gracilaria chilensis*
 382 (Rhodophyta). Ecol Evol 8:5741-5751.

383 Grzymiski J, Johnsen G, Sakshug E (1997) The significance of intracellular self-shading on
 384 the bio-optical properties of brown, red and green macroalgae. *J Phycol* 33:408-414.

385 Guillemain M-L, Faugeron S, Destombe C, Viard F, Correa JA, Valero M (2008) Genetic
 386 variation in wild and cultivated population the haploid-diploid red alga *Gracilaria*
 387 *chilensis*: how farming practices favour asexual reproduction and heterozygosity. *Evolution*
 388 62:1500-1519.

389 Guillemain M-L, Huanel OR, Martínez EA (2012) Characterization of genetic markers
 390 linked to sex determination in the haploid-diploid red alga *Gracilaria chilensis*. *J Phycol*
 391 48:365-372.

392 Guillemain M-L, Sepúlveda RD, Correa, JA, Destombe C (2013) Differential ecological
 393 responses to environmental stress in the life history phases of the isomorphic red alga
 394 *Gracilaria chilensis* (Rhodophyta). *J Appl Phycol* 25:215-224.

395 Guillemain M-L, Valenzuela P, Gaitán-Espitia JD, Destombe C (2014) Evidence of
 396 reproductive cost in the triphasic life history of the red alga *Gracilaria chilensis*
 397 (Gracilariales, Rhodophyta). *J Appl Phycol* 26:569-575.

398 McLachlan J (1973) Growth media-marine. In: Stein JR (ed) *Handbook of Phycological*
 399 *Methods*. Cambridge University Press, Cambridge, pp 25-52.

400 McLachlan J, Bird CJ (1984) Geographical and experimental assessment of the distribution
 401 of species of *Gracilaria* in relation to temperature. *Helgolander Meeresun* 38:319-334.

402 Meyer RS, Duval AE, Jensen HR (2012) Patterns and processes in crop domestication: an
 403 historical review and quantitative analysis of 203 global food crops. *New Phytol* 196:29-48.

404 Moll BA, Steinback KE (1986) Chilling sensitivity in *Oryza sativa*: the role of protein
 405 phosphorylation in protection against photoinhibition. *Plant Physiol* 80:420-423.

406 Reed TE, Waples RS, Schindler DE, Hard JJ, Kinnison MT (2010) Phenotypic plasticity
 407 and population viability: the importance of environmental predictability. *Proc R Soc B*
 408 277:3391-3400.

409 Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M (2006) Jack of all trades,
 410 master of some? On the role of phenotypic plasticity in plant invasions. *Ecol Lett* 9:981-
 411 993.

412 Robinson N, Winberg P, Kirkendale L (2013) Genetic improvement of macroalgae: status
 413 to date and needs for the future. *J. Appl Phycol* 25:703-716.

414 Saldías GS, Largier JL, Mendes R, Pérez-Santos I, Vargas CA, Sobarzo M (2016) Satellite-
 415 measured interannual variability of turbid river plumes off central-southern Chile: spatial
 416 patterns and the influence of climate variability. *Prog Oceanogr* 146:212-222.

417 Santelices B, Ugarte R (1990) Ecological differences among Chilean population of
 418 commercial *Gracilaria*. *J Appl Phycol* 2:17-26.

419 Schreiber U, Endo T, Mi H, Asada K (1995) Quenching analysis of chlorophyll
 420 fluorescence by the saturation pulse method: particular aspects relating to the study of
 421 eukaryotic algae and cyanobacteria. *Plant Cell Physiol* 36:873-882.

422 Simms EL (2000) Defining tolerance as a norm of reaction. *Evol Ecol* 14:563-570.

423 Usandizaga S, Camus C, Kappes JL, Guillemain M-L, Buschmann AH (2018) Nutrients, but
 424 not genetic diversity, affect *Gracilaria chilensis* (Rhodophyta) farming productivity and
 425 physiological responses. J Phycol 54:860-869.

426 Valero M, Guillemain M-L, Destombe C, Jacquemin B, Gachon C, Badis Y, Buschmann
 427 AH, Camus C, Faugeton S (2017) Perspectives on domestication research for sustainable
 428 seaweed aquaculture. Perspect Phycol 4:33-46.

429 Westermeier R, Gómez I, Rivera P (1993) Suspended farming of *Gracilaria chilensis*
 430 (Rhodophyta, Gigartinales) at Cariquilda river, Maullín, Chile. Aquaculture 113:215-229.

431 Wienke C, Dieck I (1989) Temperature requirements for growth and temperature tolerance
 432 of macroalgae endemic to the Antarctic region. Mar Ecol Prog Ser 54:189-197.

433 Zeder MA (2015) Core questions in domestication research. P Natl Acad Sci 11:3191-
 434 3198.

435 Zohary D (1984) Modes of evolution in plants under domestication. In: Grant W F (ed)
 436 Plant Biosystematics. Academic Press Canada, Montreal, pp 579-586.

437 Zohary D (2004) Unconscious selection and the evolution of domesticated plants. Econ
 438 Bot 58:5-10.

439

440

441 Figure Legends

442 Fig 1. Location of the three sites sampled along the Chilean coast. Photographs of farmed
443 and natural populations studied are given for each site. All photographs by S. Usandizaga.

444

445 Fig 2. Mean ($\pm$ SE) of the specific growth rate (SGR) of *Agarophyton chilensis* thalli
446 sampled from farmed and natural populations in Concepción (A and B), Maullín (C and D)
447 and Ancud (E and F). Thalli were submitted to three temperature treatments (black bars:
448 T=10°C, light grey bars: T= 15°C and dark grey bars: T= 20°C). Values are given after 30
449 days of experiment. Different letters denote significant differences between temperature
450 treatments (Tukey's hsd posthoc tests; $p < 0.05$; results given independently within each
451 sampling site and temperature treatment).

452

[Click here to view linked References](#)

1 EFFECT OF TEMPERATURE VARIATION IN *AGAROPHYTON CHILENSIS*:
2 CONTRASTING THE RESPONSE OF NATURAL AND FARMED POPULATIONS

3 Sara Usandizaga

4 Programa de Doctorado en Ciencias, mención Conservación y Manejo de Recursos
5 Naturales, Centro i~mar and CeBiB, Universidad de Los Lagos, Camino Chiquihue Km 6,
6 Puerto Montt, Chile

7 Carolina Camus

8 Centro i~mar and CeBiB, Universidad de Los Lagos, Camino Chiquihue Km 6, Puerto
9 Montt, Chile

10 José Luis Kappes

11 Centro i~mar and CeBiB, Universidad de Los Lagos, Camino Chiquihue Km 6, Puerto
12 Montt, Chile

13 Marie-Laure Guillemin

14 Centro FONDAP de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes
15 (IDEAL), Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias,
16 Universidad Austral de Chile, Valdivia, Chile.

17 CNRS, Sorbonne Université, Pontificia Universidad Católica de Chile, Universidad Austral
18 de Chile, UMI Evolutionary Biology and Ecology of Algae, Station Biologique de Roscoff,
19 29680 Roscoff, France.

20 Alejandro H. Buschmann²

21 Centro i~mar and CeBiB, Universidad de Los Lagos, Camino Chiquihue Km 6, Puerto
22 Montt, Chile

23 ¹ Date of submission and acceptance

24 ² Author for correspondence: Alejandro H. Buschmann, e-mail: abuschma@ulagos.cl,
25 phone: +56 65 2322423

26 Running title: *Agarophyton chilensis* response to temperature variation

27 **Abstract**

28 During the domestication process, farmers influence the reproduction and care of organisms
29 to ensure a predictable supply of the resource of interest, causing changes in phenotypic
30 and genotypic character frequencies. In Chile, as a result of unconscious selection and
31 domestication process, farmed populations of the red alga *Agarophyton chilensis* have most
32 likely undergone a reduction in genetic diversity and a modification in life-history traits
33 compared to wild populations. In order to understand the implications that these processes
34 may have in *A. chilensis*, in this study we investigated how temperature variations (10 °C,
35 15 °C and 20 °C) affect growth and photosynthetic responses of natural and farmed
36 populations from three different localities along the Chilean coast. Natural population's
37 growth decreased at low and high temperature levels while all three farmed populations
38 respond in a very similar way to temperature variation. We propose that a possible outcome
39 of farming, in the *A. chilensis* vegetatively propagated crops, could have been the selection
40 of general-purpose-genotypes able to perform adequately across the range of temperature
41 tested in our experiment. Furthermore, our results showed that photosynthetic activity was
42 also affected by temperature treatments (e.g., different maximum maximal electron

transport rate and quantum yield values depending on the population type and temperature).

In a context of climate change, *A. chilensis* farmed populations may be better able to cope with impacts of anthropogenic activities than natural populations due to the buffer effect of their general-purpose-genotypes, tolerant to a wide range of conditions.

Key index words: abiotic factor, domestication, management, general-purpose-genotypes, photosynthesis, origin, seaweed, selection.

Abbreviations: ETR_{max} , maximal electron transport rate; F_v/F_m , maximal quantum yield; E_k , irradiance of saturation of ETR; NPQ_{max} , maximal non-photochemical quenching; PAR, photosynthetically active radiation; PSII, photosystem II; RLC, rapid light curves.

Introduction

Domestication is considered a long and complex process during which domesticators influence the reproduction and care of domesticated species to guarantee predictable supply of resources presenting selected traits of interest for human use (Zeder, 2015). This selection process generates changes in phenotypic and genotypic character frequencies of cultivated populations (Zohary, 1984). Even if domestication of marine species is much more recent than the one of terrestrial animals and plants (Duarte et al. 2007), strong evidence for domestication has been found in a few cultivated seaweeds (Valero et al. 2017). As for terrestrial plants (Meyer et al. 2012), some domesticated seaweeds are characterized by a shift in their reproductive strategy (e.g., changes from sexual reproduction to vegetative propagation) between natural and farmed populations (Valero et al. 2017). This shift in reproductive strategy has been demonstrated for Agarophyton (referred as *Gracilaria* in Guillemain et al. 2008) and is probably also present in

65 *Kappaphycus* (Ask and Azanza 2002). Asexual propagation enables farmers to selectively
66 multiply superior genotypes and maintain desired phenotypes through vegetative
67 propagation (Valero et al. 2017).

68 In Chile, intensive seaweed farming is limited to the domesticated red alga *Agarophyton*
69 *chilensis* used mainly for agar extraction (Buschmann et al. 2017). Even if the
70 domestication process of this species has begun only a few decades ago, the almost
71 complete predominance of diploid individuals in farms demonstrate that farming practices
72 had significantly modified life-history traits as compared to wild populations (Guillemin et
73 al. 2008). Moreover, recent investigations have demonstrated that this red alga colonized
74 the Chilean coast from New Zealand, likely at the end of the Last Glacial Maximum
75 (Guillemin et al. 2014). The lower genetic diversity of the Chilean populations, when
76 compared to the ones from New Zealand, is indeed consistent with a genetic bottleneck
77 resulting from a transpacific range extension and was probably reinforced by the over-
78 exploitation of natural Chilean populations during the 90's (Guillemin et al. 2014). In
79 Chile, active transport and exchange of inoculums of *A. chilensis* between coastal
80 communities of fishermen for cultivation purposes have contributed to the artificial
81 expansion of the species distribution. Nowadays, natural populations are distributed
82 between 30°S to 45°S while farming extends further north, up to Antofagasta, 17°S (Bird et
83 al. 1986, Guillemin et al. 2008). Human activities have also led to a loss of genotypic
84 diversity in farmed populations that could be partially linked to involuntary selection of
85 faster growing thalli, during the first steps of the domestication process (Guillemin et al.
86 2008, Guillemin et al. 2013, Valero et al. 2017).

87 Reduced genetic diversity can severely affect the ability of populations to resist pests and
88 pathogens and limit the scope for future genetic improvement in domesticated crops
89 (Robinson et al. 2013). Besides, it has been reported that a reduction in genetic diversity
90 could lead to lower growth and resilience in highly stressful and/or variable environment
91 (Simms 2000). However, to date, few studies have focused on the potential
92 ecophysiological differences between natural and farmed populations of A. chilensis.
93 Gallegos-Sánchez et al. (2018), detected a significant and negative effect of low salinity
94 conditions on thalli sampled from both natural and farmed A. chilensis populations but with
95 farmed population's thalli being less affected than the natural ones. Results suggested that
96 farmed populations might be more tolerant to salt stress than wild ones in this species and
97 the authors proposed that this difference between population types could be due to previous
98 selective process carried out by farmers.

99 Considering the possible consequences of unconscious selection (as defined in Zohary
100 2004) and domestication on GAgarophyton chilensis, we propose that natural populations
101 will be less sensitive to temperature variations than farmed populations. Genotypic
102 diversity has been shown to be higher in natural than farmed A. chilensis Chilean
103 populations and we propose that the farms will show less effective mechanisms of
104 acclimation (e.g., enhancing of photosynthetic performance) than the one observed in
105 natural populations when confronted with temperature considered as high in their natural
106 environment (i.e., water of the southern coast of Chile do not generally reach 20°C;
107 Westermeier et al. 1993). The aim of the present study was to assess the effect of
108 temperature on growth and photosynthetic responses of both natural and farmed stands of
109 A. chilensis and tetrasporophyte thalli sampled from three sites along the Chilean coast

were followed during one month in controlled laboratory conditions at 10°C, 15°C and 20°C.

Materials and methods

Study sites and life cycle phase determination of the sampled thalli

A total of 600 individuals were sampled from 3 natural and 3 farmed populations (i.e., 100 individuals in each population) during a spring season. One farmed and one natural population were sampled from three sites: Concepción, Maullín and Ancud (Fig.1). The distinction between natural and farmed populations was first based on whether *Agarophyton chilensis* thalli were actively planted or not by farmers. As previously reported for the species (Guillemin et al. 2008), thalli were attached to small rocks and pebbles by a holdfast in the three natural *A. chilensis* beds while unattached thalli were found embedded in the sandy bottom in the three farmed stands. In each site, natural and farmed populations were separated by 1 km approximately. In natural beds, individuals sampled correspond to distinct holdfasts (i.e., distinct genotypes produced by sexual reproduction and spore settlement). In farms, to avoid sampling fragments of the same asexually propagated genotype, sampled thalli were separated by at least by 2 m. All the collected thalli were transported in isolated boxes to the CEACIMA hatchery (Centro de Investigación de Acuicultura y Ciencias del Mar, Universidad de Los Lagos) located in the Metri Bay (41°36'S, 72°43'W). Once in the hatchery, each thallus was cleaned with fresh water and all epiphytes were removed by hand. Thalli were individually marked with a numbered tag and maintained in 400 L tanks at 12°C with constant aeration, 12L:12D photoperiod, 20 $\mu\text{mol electron m}^{-2} \text{ s}^{-1}$ photon flux and weekly filtered seawater exchange.

Collected thalli were observed under stereoscope microscope (Stemi DV4, Zeiss, Jena, Germany) to determine phase (i.e., diploid tetrasporophytes or haploid gametophytes) of mature individuals. For vegetative individuals, a 3-cm fragment of tissue was excised from each thallus, placed into plastic bags with silica gel for rapid dehydration. The sex markers available for *A. chilensis* were amplified following Guillemín et al. (2012) and the amplification products were visualized in 1.5 % agarose gel (w/v) after adding 2 µl of GelRed™ (Biotium, Fremont, USA). Results were used to determine sex and phase of vegetative individuals. In order to prevent experimental bias due to ecophysiological variability between life cycle phases (Guillemín et al. 2013), only diploid tetrasporophytes were selected for our experiments.

Experimental design

The experimental design consisted of 90 2-L Erlenmeyer flasks (i.e., 6 populations of origin of thalli x 3 temperature treatments x 5 replicates per population per temperature treatment) arranged in 15 60-L plastic water tanks fitted with temperature control systems. Three temperature treatments were used: 10°C, 15°C and 20°C. These temperatures were chosen since they roughly represent the temperature range encountered in the field by the study species. Temperature conditions varied widely within sites where *A. chilensis* is found and between regions populated by the species along the Chilean coasts with values between 9 °C and 16 °C recorded in Maullín (Westermeyer et al. 1993) and between 10 °C and 20°C in areas further north such as Concepción, Coquimbo and Antofagasta (Santelices and Ugarte 1990). In each 60-L plastic water tanks, one 50W automatic heater (Whale VK-1000, Regent) and one stainless steel thermometer (Hagen, Phelan) were used to maintain constant temperature. For each population under study, five replicates (i.e., five 2-L flasks)

were followed per temperature treatment and eight thalli (5 cm length each), selected randomly from distinct tetrasporophytes, were placed in each 2-L flask. Thalli were selected without replacement from a pool of 40 tetrasporophytes, available for each population of origin (see above). Once a week, thalli were transferred to clean 2-L flasks with fresh Provasoli culture media (McLachlan 1973). After one week of acclimation at 12°C, the laboratory experiment was run during 30 days. All Erlenmeyer flasks were under constant conditions of aeration, photoperiod (12 h light: 12 h dark) and photon flux (20 $\mu\text{mol electron m}^{-2} \text{ s}^{-1}$).

Growth

Fresh weight of each thallus was assessed weekly on an analytical balance (Sartorius TE 313 DS, Germany) and the specific growth rate (SGR) was calculated as the percentage of wet weight gain per day according to the formula: $\text{SGR} = [\ln (W_f \cdot W_i^{-1}) / (t_f - t_i)] \times 100$; where W_i = initial fresh weight, W_f = final fresh weight, and t = time (days). Initial fresh weight in the 2-L flasks was of 0.12 ± 0.06 g, some variation in fresh weight exist between each 2-L flask at the beginning of the experiment since calibration of algal material was based on thallus length (see above).

Physiological variables

Thallus pieces were collected at the end of the experiment to measure rapid light curves (RLC). As an indicator of quantum efficiency and photoinhibition, we used, F_v/F_m , which was determined after incubation of 20 min of the thalli in darkness (Schreiber et al. 1995) with a Junior PAM (Walz GmbH, Effeltrich, Germany). The electron transport rate (ETR, $\mu\text{mol electrons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) was determined after 20 s exposure in 12 increasing intensities of

PAR (up to 1500 $\mu\text{mol photon} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) provided by a blue light of the Junior PAM device (Schreiber et al. 1995). ETR was calculated according to Schreiber et al. (1995) as follows:

$$\text{ETR} = \Delta F/F'_m \cdot E \cdot A \cdot F_{\text{II}} \text{ (}\mu\text{mol electrons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\text{)} \quad (1)$$

Where $\Delta F/F'_m$ is the effective quantum yield, E is the incident PAR (photosynthetically active radiation) irradiance expressed in $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, A is the thallus absorptance and F_{II} is the fraction of chlorophyll related to photosystem II (PSII, 400-700nm), being 0.15 in red seaweed (Grzyski et al. 1997). As an estimator of photosynthetic efficiency, the initial slope of ETR (α_{ETR}) and maximum ETR (ETR_{max}) were obtained from the tangential function reported by Eilers and Peeters (1988) and the irradiance saturation ($E_{k_{\text{ETR}}}$) was calculated from the intercept between these two parameters. Representing potential thalli photoprotective mechanism, non-photochemical quenching (NPQ) was measured according to Schreiber et al. (1995). The maximal non-photochemical quenching (NPQ_{max}) was calculated from the tangential function of NPQ versus irradiance function according to Eilers and Peeters (1988).

Statistical treatment

All analyses were performed in R (3.2.4 version) (Cayuela 2011). Assumptions of homogeneity of variances and normal distribution were tested using Levene's test and Shapiro-Wilk, respectively. When non-normal residuals and heteroscedasticity were detected, the data were transformed using logarithm (for E_k and SGR) or Box-cox (for F_v/F_m). The experimental design fitted a three-way ANOVA with treatment (temperature: 10°C, 15°C and 20°C), site of origin (Concepción, Maullín and Ancud) and population type

(natural or farmed) considered as fixed factors. Statistical differences between groups were analyzed using comparisons of means (Tukey's HSD). Significances were set at $p < 0.05$.

Results

Significant differences were detected in *Agarophyton chilensis* specific growth rate (SGR) after 30 days of experimentation between temperature treatments ($F_{(2, 72)} = 15.46$; $P < 0.0001$), between population types ($F_{(1, 72)} = 4.93$; $P = 0.03$) and a significant interaction was also detected between population type and site of origin ($F_{(2, 72)} = 5.00$; $P = 0.009$). In Maullín and Ancud, no significant differences between temperature treatments were detected for farmed thalli (Fig. 2C and E), while farmed thalli from Concepción showed a slightly but significantly lower SGR at 20°C than at 15°C (7.26 ± 1.48 and 9.45 ± 1.92 g · day⁻¹, respectively; Fig. 2A). In addition, differences among Concepción farmed and natural populations were observed (Tukey test; $P < 0.05$, see Figure 2A and B). In contrast, SGR was significantly higher at 15°C for thalli sampled in natural *A. chilensis* stands, whatever the site under study (SGR at 15°C: 7.49 ± 2.07 g · day⁻¹ in Concepción, 8.81 ± 2.60 g · day⁻¹ in Maullín and 8.67 ± 0.84 g · day⁻¹ in Ancud; Fig. 2B, D and F). Finally, the growth rate did not decrease significantly (Tukey test; $P < 0.05$) at the lower (10 °C) and the higher (20 °C) temperature tested for Maullín and Ancud populations (Fig. 2 C, D, E and F).

Regarding the photosynthetic responses, significant effect of temperature treatments were detected for all parameters measured: the optimal quantum yield of fluorescence (F_v/F_m ; $F_{(2, 35)} = 13.20$; $P < 0.00001$), the maximal electron transport rate (ETR_{max} ; $F_{(2, 35)} = 6.17$; $P = 0.02$), the saturation irradiance (E_k ; $F_{(2, 35)} = 6.97$; $P = 0.04$) and the maximal non-photochemical quenching (NPQ_{max} ; $F_{(2, 35)} = 6.02$; $P = 0.01$) (see Table 1). Interactions

between temperature treatment and population type were also observed for ETR_{max} ($F_{(2,35)}=9.57$; $P=0.0005$) and Ek ($F_{(2,35)}=5.34$; $P=0.01$) (Table 1). Values of ETR_{max} and Ek were significantly higher for thalli sampled in the farmed population of Concepción and grown at 20°C than for thalli sampled in the farmed population of Ancud and grown at 10°C (18.63 ± 12.76 and $1.44 \pm 0.37 \mu\text{mol electrons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 442.97 ± 306.60 and $31.86 \pm 4.78 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, for ETR_{max} and Ek , respectively; Tukey's HSD tests; $p < 0.05$; Table 2).

Significant interactions between temperature treatment, population type and site of origin were observed for F_v/F_m ($F_{(4,35)}=2.86$; $P=0.04$) and NPQ_{max} ($F_{(4,34)}=3.31$; $P=0.02$) (Table 1). In Maullín at 15°C, values of NPQ_{max} were significantly higher for thalli sampled in the farmed bed than the natural population (Tukey's HSD tests; $p < 0.05$; Table 2).

Discussion

The present study confirms that natural and farmed *Agarophyton chilensis* populations respond differentially to temperature variations. Indeed, contrarily to our expectations, our results indicate that farmed populations are less sensitive to temperature variations than natural ones (i.e., thalli growth rate are mostly similar at 10°C, 15°C and 20°C for farms while thalli from natural populations have a higher growth rate at 15°C). In Chile, farmed population has been under unconscious human selection pressure for, at least, three decades and we propose that a possible outcome could have been the selection of general-purpose-genotypes (as in Baker 1974) in these vegetatively propagated crops. Our results also showed that photosynthetic activity was affected by temperature treatments (e.g., different maximum maximal electron transport rate and quantum yield values depending on the population type and temperature).

Regardless of the origin of the natural population under study, our experiment shows that thalli specific growth rate (SGR) was higher at 15°C than at lower (i.e., 10°C) or higher (i.e., 20°C) temperatures. Even if SGR tended to be much more homogeneous for farmed thalli at all temperatures, one slight difference was observed for the farmed thalli from Concepción that show significantly less growth at 20°C. Supporting our results, a study realized by Santelices and Ugarte (1990) on *A. chilensis* (as *Gracilaria chilensis*) natural populations from Maullín, also revealed better growth rates at 15°C than at 10°C or 20°C. Most temperate species of the genus *Agarophyton* has been shown to grow faster in temperature ranging between 15° and 20°C (McLachlan and Bird 1984). A study performed in *Gracilaria gracilis* (as *G. verrucosa*) reported a slow growth rate at 10°C and high mortality after a 14 days' heat-wave in Saldanha Bay, South Africa (Engledow and Bolton 1992). *A. chilensis*, is a temperate-water species with a distribution limited to southern part of the Pacific (Bird et al. 1986, Guillemin et al. 2008) and it is possible that the species present a metabolism with limited temperature tolerance.

Although farming has begun only a few decades ago (i.e., during the 80's) in Chile, the predominant mechanism for stock propagation by cuttings used in *Agarophyton chilensis* farms has already significantly decreased their genotypic diversity when compared with wild populations (Guillemin et al. 2008). There is an overall agreement that genetically more variable populations may be associated with higher resilience, increased productivity and population growth rate as compared with less variable populations (Forsman 2014). However, contrasting with our expectations, farmed thalli in our experiment clearly show a strong ability to grow in contrasting environmental conditions, including the quite “extreme” temperature of 20°C tested. In the same way, Gallegos-Sanchez et al. (2018)

265 concluded that *A. chilensis* farmed populations may be less sensitive to salt stress and able
266 to grow in a greater range of salinity than natural populations. One possible explanation for
267 these results is that farmed populations of *A. chilensis* are composed mostly of general-
268 purpose genotypes, able to grow in highly stressful and/or variable environments. General-
269 purpose-genotypes are sometime also referred as ‘Jack-of-all-trades, master of none’ since
270 they are described as versatile genotypes that are able to perform adequately across a range
271 of environments but are not superior in any of them. These general-purpose-genotypes can
272 confer a species or population a broad tolerance to environmental changes and are often
273 associated with species invasion (Baker 1974, Richards et al. 2006). In Chile, farms have
274 been developed using material growing embedded in muddy estuaries and sandy bays.

275 These habitats are typically highly heterogeneous and present strong seasonal variations in
276 temperature and salinity (Westermeyer et al. 1993, Buschmann et al. 1995). It have been
277 demonstrated that intraspecific competition for resources utilization in clonal individuals
278 living in habitats characterized by fluctuating environmental conditions could lead to
279 positive selection of general-purpose-genotypes (Arnaud-Haond et al. 2012).

280 In benthic algae, temperature variations affect photosynthetic metabolism (Davison 1991)
281 determining, for example, seasonal distribution (De Nicola 1996). However, habitats
282 characterized by strong spatial and temporal variations of abiotic factors, request constant
283 adjustment of photosynthetic processes in species populating them (Ensminger et al. 2001).

284 In our study, the highest values of ETR_{max} and E_k were observed in the *A. chilensis* farmed
285 population from Concepción grown at 20°C. These results suggest that the effect of high
286 temperatures on photosynthetic metabolism of this farmed population could be mild.
287 Driven by seasonal changes in river discharge, precipitation, and coastal upwelling, high
288 variability in abiotic conditions (e.g., temperature, salinity and turbidity; Saldías et al.

2016) has been observed in the river mouths where *A. chilensis* thalli are planted in Concepción. These characteristics could be associated to distinctive heat susceptibility of the photosynthetic metabolism of the *A. chilensis* thalli growing in Concepción farms. In our experiment, the response of the maximum quantum yield (F_v/F_m) to temperature was quite variable. The lowest value of F_v/F_m (indicating photoinhibition) was observed at intermediate temperature (15°C) in the farm of Maullín. In plants, it is well known that limitation of electron transport that reduces the ability of plants to use light result in an excess light energy that may cause photoinhibition due to damage to the PSII apparatus (Moll and Steinback 1986). However, short-term response of photosynthesis to temperature cannot easily be used to infer the long-term response of algal growth (Wienke and Dieck 1989). Indeed, despite the possible signal of photoinhibition detected at 15°C, no limited growth could be observed in these *Agarophyton-chilensis* thalli. Populations of *Agarophyton-chilensis* from different sites in Chile have been reported to present ecological differences, potentially linked to local adaptation in response to specific abiotic and biotic environmental characteristics (Santelices and Ugarte 1990, Usandizaga et al. 2018). The success of *Agarophyton* farming depends in part on the origin of the initial inoculum since differences in thallus morphology, agar yield and gel strength and susceptibility to epiphytes exist among regions and populations. Indeed, it have been suggested that random transplantation between distinct habitats could lead to cultivation failure (Santelices and Ugarte 1990). However, in our experiment, even if we included sampling sites located more than 700 km apart, no major effect of the site of origin were detected on growth or photosynthesis. A possible explanation for this discrepancy is that continuous transplantations and exchanges during the last decades have leaded to the homogenization of the genetic diversity among the whole Chilean coast. However, this

hypothesis is not in accordance with population genetic studies showing the presence of clear genetic divergence between regions in Chile (Guillemin et al. 2008 and 2014). Studies focused on the effect of other stressors (e.g., nutrient supply, salinity, irradiance) and the cumulative effects of various of these stressors on the physiological responses of distinct genotypes is now needed in order to better explore the resistance of *A. chilensis* populations to stress.

Conclusion

Agarophyton chilensis tolerance of a wide range of abiotic conditions has been proposed as one of the main reasons of the species successful expansion in the Pacific and establishment in a wide array of habitats (Santelices and Ugarte 1990, Chow et al. 2001). These successful extension waves have probably also been facilitated by intrinsic characteristics of the species, such as its capacity to shift between sexual and asexual reproduction (Guillemin et al. 2014). We suggest that the possible selection for general-purpose-genotypes in the asexually reproducing farmed populations may help modulating the impact of environmental variation on population dynamics (Reed et al. 2010) and Chilean *Agarophyton* crop to better cope with impacts of climate change and direct anthropogenic activities. Nevertheless, implementation of breeding strategy and cultivar selection for mariculture systems improvement has not yet begun in Chile and development of long-term management plans for the sustainable exploitation of *A. chilensis* populations is dearly needed.

Acknowledgements

This research was supported by a doctoral fellow from Universidad de Los Lagos

(Chile) to SU, CeBiB (CONICYT, FB-0001) to AHB and CC and Fondecyt N°1150987 and N°1170541 (CONICYT) to AHB and MLG, respectively. Additional support was provided by the grant “Término de Tesis” awarded by Universidad de Los Lagos. The authors are grateful for the help in the field of R. Altamirano and S. V. Pereda for the revision of this manuscript. We also wish to thank two anonymous referees who greatly improved the manuscript with constructive suggestions. The authors declare no conflict of interest.

References

Arnaud-Haond S, Duarte C, Diaz-Almela E, Marbà N, Sintes T, Serrão (2012) Implications of extreme life span in clonal organisms: millenary clones in meadows of the threatened seagrass *Posidonia oceanica*. PloS one 7:e30454.

Ask EI, Azanza RV (2002) Advances in cultivation technology of commercial eucheumatoid species: a review with suggestions for future research. Aquaculture 206:257-277.

Baker HG (1974) The evolution of weeds. Annu Rev Ecol Syst 5:1-24.

Bird CJ, McLachan J, Oliveira EC (1986) *Gracilaria chilensis* sp. nov; (Rhodophyta, Gigartinales), from Pacific South America. Can J Bot 64:2928-2934.

~~Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Adv Genet 13:115-155.~~

~~Buschmann AH, Correa JA, Westermeier R, Hernandez-Gonzalez MDC, Norambuena R (2001) Red algal farming in Chile: a review. Aquaculture 194:203-220.~~

Buschmann AH, Westermeier R, Retamales C (1995) Cultivation of *Gracilaria* in the sea-bottom in southern Chile: a review. J Appl Phycol 7:291–301.

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

Cayuela L (2011) Modelos lineales: Regresión, ANOVA y ANCOVA. Universidad Rey Juan Carlos, Madrid.

Chow FY, Macchiavello J, Cruz SS, Fonck E, Olivares J (2001) Utilization of *Gracilaria chilensis* (Rhodophyta: Gracilariaceae) as a biofilter in the depuration of effluents from tank cultures of fish, oysters, and sea urchins. J World Aquac Soc 32:215-220.

Davison IR (1991) Environmental effects on algal photosynthesis: temperature. J Phycol 27:2–8.

De Nicola DM (1996) Periphyton responses to temperature at different ecological levels. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego, pp 149-181.

Duarte CM, Marbá N, Holmer M (2007) Rapid domestication of marine species. Science 316:382.

Eilers PHC, Peeters JCH (1988) A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. Ecol Model 42:199-215.

~~Engel CR, Åberg P, Gaggiotti O, Destombe C, Valero M (2001) Population dynamics and stage structure in a red haploid-diploid red seaweed, *Gracilaria gracilis*. J Ecol 89:436-450.~~

382 Engledow HR, Bolton JJ (1992) Environmental tolerances in culture and agar content of
 383 *Gracilaria verrucosa* (Hudson) Papenfuss (Rhodophyta, Gigartinales) from Saldanha Bay.
 384 S Afr J Bot 58:263-267.

385 Ensminger I, Xyländer M, Hagen C, Braune W (2001) Strategies providing success in a
 386 variable habitat: III. Dynamic control of photosynthesis in *Cladophora glomerata*. Plant
 387 Cell Environ 24:769-779.

388 Forsman A (2014) Rethinking phenotypic plasticity and its consequences for individuals,
 389 populations and species. Heredity 115:1–9.

390 Gallegos-Sánchez CF, Beltrán J, Flores V, González AV, Santelices B (2018) Testing the
 391 effects of heterozygosity on growth rate plasticity in the seaweed *Gracilaria chilensis*
 392 (Rhodophyta). Ecol Evol 8:5741-5751.

393 Grzyski J, Johnsen G, Sakshug E (1997) The significance of intracellular self-shading on
 394 the bio-optical properties of brown, red and green macroalgae. J Phycol 33:408-414.

395 Guillemin M-L, Faugeron S, Destombe C, Viard F, Correa JA, Valero M (2008) Genetic
 396 variation in wild and cultivated population the haploid-diploid red alga *Gracilaria*
 397 *chilensis*: how farming practices favour asexual reproduction and heterozygosity. Evolution
 398 62:1500-1519.

399 Guillemin M-L, Huanel OR, Martínez EA (2012) Characterization of genetic markers
 400 linked to sex determination in the haploid-diploid red alga *Gracilaria chilensis*. J Phycol
 401 48:365-372.

402 Guillemin M-L, Sepúlveda RD, Correa, JA, Destombe C (2013) Differential ecological
 403 responses to environmental stress in the life history phases of the isomorphic red alga

404 *Gracilaria chilensis* (Rhodophyta). J Appl Phycol 25:215-224.

405 Guillemín M-L, Valenzuela P, Gaitán-Espitia JD, Destombe C (2014) Evidence of
 406 reproductive cost in the triphasic life history of the red alga *Gracilaria chilensis*
 407 (Gracilariales, Rhodophyta). J Appl Phycol 26:569-575.

408 ~~Kim JK, Yarish C, Hwang EK, Park M, Kim Y (2017) Seaweed aquaculture: cultivation~~
 409 ~~technologies, challenges and its ecosystem services. Algae 32:1-13.~~

410 ~~Krueger-Hadfield SA, Kollars NM, Byers JE, Greig TW, Hammann M, Murray DC,~~
 411 ~~Murren CJ, Strand AE, Terada R, Weinberger F, Sotka EE (2016a) Invasion of novel~~
 412 ~~habitats uncouples haplo-diplontic life cycles. Mol Ecol 25:3801-3816.~~

413 ~~Krueger-Hadfield, SA, Carmona GH, Terada R, López-Vivas JM, Riosmena-Rodríguez R~~
 414 ~~(2016b) New Record of the non-Native Seaweed *Gracilaria parvispora* in Baja California—~~
 415 ~~A Note on Vergara-Rodarte *et al.* (2016). Cryptogam Algal 37:257-263.~~

416 ~~Krueger-Hadfield, SA, Magill CL, Bunker F St PD, Mieszkowska N, Sotka EE, Maggs CA~~
 417 ~~(2017) When invaders go unnoticed: The case of *Gracilaria vermiculophylla* in the British~~
 418 ~~Isles. Cryptogam Algal 38:379-400.~~

419 ~~Lynch M (1984) Destabilizing hybridization, general-purpose genotypes and geographic~~
 420 ~~parthenogenesis. Quart Rev Biol 59:257-290.~~

421 McLachlan J (1973) Growth media-marine. In: Stein JR (ed) Handbook of Phycological
 422 Methods. Cambridge University Press, Cambridge, pp 25-52.

423 McLachlan J, Bird CJ (1984) Geographical and experimental assessment of the distribution
 424 of species of *Gracilaria* in relation to temperature. Helgolander Meeresun 38:319-334.

Meyer RS, Duval AE, Jensen HR (2012) Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *New Phytol* 196:29-48.

~~Miner BG, Sultan SE, Morgan SG, Padilla DK, Relyea RA (2005) Ecological consequences of phenotypic plasticity. *Trends Ecol Evol* 20:685-692.~~

~~Mitton JB, Grant MC (1984) Associations among protein heterozygosity, growth rate, and developmental homeostasis. *Annu Rev Ecol Syst* 15:479-499.~~

Moll BA, Steinback KE (1986) Chilling sensitivity in *Oryza sativa*: the role of protein phosphorylation in protection against photoinhibition. *Plant Physiol* 80:420-423.

~~Necchi OJ (2006) Short-term responses of photosynthesis to temperature and irradiance in the freshwater red alga *Compsopogon coeruleus*. *Algol Stud* 119:125-138.~~

Reed TE, Waples RS, Schindler DE, Hard JJ, Kinnison MT (2010) Phenotypic plasticity and population viability: the importance of environmental predictability. *Proc R Soc B* 277:3391-3400.

Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol Lett* 9:981-993.

Robinson N, Winberg P, Kirkendale L (2013) Genetic improvement of macroalgae: status to date and needs for the future. *J. Appl Phycol* 25:703-716.

Saldías GS, Largier JL, Mendes R, Pérez-Santos I, Vargas CA, Sobarzo M (2016) Satellite-measured interannual variability of turbid river plumes off central-southern Chile: spatial patterns and the influence of climate variability. *Prog Oceanogr* 146:212-222.

~~Santelices B, Doty MS (1989) A review of *Gracilaria* farming. *Aquaculture* 78:95-133.~~

447 Santelices B, Ugarte R (1990) Ecological differences among Chilean population of
 448 commercial *Gracilaria*. J Appl Phycol 2:17-26.

449 ~~Schmalhausen I (1949) Factors of evolution. Blakiston Press, New York, pp 326.~~

450 Schreiber U, Endo T, Mi H, Asada K (1995) Quenching analysis of chlorophyll
 451 fluorescence by the saturation pulse method: particular aspects relating to the study of
 452 eukaryotic algae and cyanobacteria. Plant Cell Physiol 36:873-882.

453 Simms EL (2000) Defining tolerance as a norm of reaction. Evol Ecol 14:563-570.

454 Usandizaga S, Camus C, Kappes JL, Guillemain M-L, Buschmann AH (2018) Nutrients, but
 455 not genetic diversity, affect *Gracilaria chilensis* (Rhodophyta) farming productivity and
 456 physiological responses. J Phycol 54:860-869. ~~<https://doi.org/10.1111/jpy.12785>~~.

457 Valero M, Guillemain M-L, Destombe C, Jacquemin B, Gachon C, Badis Y, Buschmann
 458 AH, Camus C, Faugeton S (2017) Perspectives on domestication research for sustainable
 459 seaweed aquaculture. Perspect Phycol 4:33-46.

460 ~~Via S, Gomulkiewicz R, De Jong G, Scheiner S, Schlichting CD, Tienderen PV (1995)~~
 461 ~~Adaptive phenotypic plasticity: consensus and controversy. Trends Ecol Evol 10:212-217.~~

462 Westermeier R, Gómez I, Rivera P (1993) Suspended farming of *Gracilaria chilensis*
 463 (Rhodophyta, Gigartinales) at Cariquilda river, Maullín, Chile. Aquaculture 113:215-229.

464 Wienke C, Dieck I (1989) Temperature requirements for growth and temperature tolerance
 465 of macroalgae endemic to the Antarctic region. Mar Ecol Prog Ser 54:189-197.

466 Zeder MA (2015) Core questions in domestication research. P Natl Acad Sci 11:3191-
467 3198.

468 Zohary D (1984) Modes of evolution in plants under domestication. In: Grant W F (ed)
469 Plant Biosystematics. Academic Press Canada, Montreal, pp 579-586.

470 [Zohary D \(2004\) Unconscious selection and the evolution of domesticated plants. Econ](#)
471 [Bot 58:5-10.](#)

472

473

474 Figure legends

475 Fig 1. Location of the three sites sampled along the Chilean coast. Photographs of farmed
476 and natural populations studied are given for each site. All photographs by S. Usandizaga.

477

478 Fig 2. Mean ($\pm$ SE) of the specific growth rate (SGR) of *Agarophyton chilensis* thalli
479 sampled from farmed and natural populations in Concepción (A and B), Maullín (C and D)
480 and Ancud (E and F). Thalli were submitted to three temperature treatments (black bars:
481 T=10°C, light grey bars: T= 15°C and dark grey bars: T= 20°C). Values are given after 30
482 days of experiment. Different letters denote significant differences between temperature
483 treatments (Tukey's hsd posthoc tests; $p < 0.05$; results given independently within each
484 sampling site and temperature treatment).

485

Table 1. Results of ANOVA analyses on of the specific growth rate (SGR) and photosynthetic parameters for *Aequorophyton chilensis* thalli submitted to three temperature treatments during one month. Temperature treatment (T.T; 10°C, 15°C and 20°C), site of origin (S; Concepción, Maullín and Ancud) and population type (P; natural or farmed) were considered as fixed factors. Bold: significant at: *, p<0.05, **, p<0.01, ***, p<0.001.

	Temperature Treatment (T.T)			Population type (P)			Site of origin (S)			Interaction (P x T.T)			Interaction (S x T.T)			Interaction (P x S)			Interaction (T.T x P x S)									
	Sumsq	df	F	p	Sumsq	df	F	p	Sumsq	df	F	p	Sumsq	df	F	p	Sumsq	df	F	p	Sumsq	df	F	p				
F_v/F_m	0.00	2	13.20	<0.01***	0.00	1	1.89	0.18	0.00	2	1.32	0.28	0.00	2	10.83	<0.01***	0.00	4	2.87	0.04*	0.00	2	0.88	0.42	0.00	4	2.86	0.04*
ETR _{max}	326.2	2	6.17	<0.01***	52.9	1	2.00	0.17	1.61	2	0.03	0.97	506	2	9.57	<0.01***	270	4	2.55	0.06	79.3	2	1.50	0.24	109.9	4	1.04	0.40
E_k	6.34	2	6.97	<0.01**	0.16	1	0.35	0.56	1.86	2	2.04	0.14	4.86	2	5.34	<0.01**	1.46	4	0.80	0.53	0.57	2	0.63	0.54	1.01	4	0.55	0.70
NPQ _{max}	0.45	2	6.02	<0.01**	0.40	1	10.68	<0.01**	0.01	2	0.16	0.85	0.11	2	1.48	0.24	0.26	4	1.73	0.16	0.23	2	3.10	0.06	0.49	4	3.31	0.02*

Table 2. Photosynthetic parameters measured in *Agarophyton chilensis* natural and farmed thalli submitted to three temperature treatments. Thalli from one natural and one farmed populations were sampled in three sites (i.e. Concepción, Maullín and Ancud).

Data are given as mean $\pm$ S.E.D (n = 5). Distinct uppercase letters denote significant differences after Tukey test.

CONCEPCIÓN			MAULLÍN			ANCUD		
T	P	Means $\pm$ S.E.	T	P	Means $\pm$ S.E.	T	P	Means $\pm$ S.E.
<i>F_v/F_m</i>								
10°C	Natural	0.60 $\pm$ 0.01 ^{ab}	10°C	Natural	0.64 $\pm$ 0.03 ^{ac}	10°C	Natural	0.66 $\pm$ 0.01 ^{ac}
15°C		0.64 $\pm$ 0.02 ^{abc}	15°C		0.37 $\pm$ 0.36 ^b	15°C		0.64 $\pm$ 0.02 ^{abc}
20°C		0.66 $\pm$ 0.02 ^{ac}	20°C		0.66 $\pm$ 0.01 ^{ac}	20°C		0.66 $\pm$ 0.01 ^{abc}
10°C	Farmed	0.68 $\pm$ 0.02 ^c	10°C	Farmed	0.69 $\pm$ 0.00 ^c	10°C	Farmed	0.66 $\pm$ 0.01 ^{ac}
15°C		0.61 $\pm$ 0.02 ^{ab}	15°C		0.61 $\pm$ 0.02 ^{ab}	15°C		0.64 $\pm$ 0.02 ^{abc}
20°C		0.64 $\pm$ 0.03 ^{abc}	20°C		0.63 $\pm$ 0.01 ^{abc}	20°C		0.65 $\pm$ 0.02 ^{abc}

ETR _{max}	10°C	Natural	5.33 $\pm$ 0.39 ^{ab}	10°C	Natural	5.94 $\pm$ 3.28 ^{ab}	10°C	Natural	3.06 $\pm$ 1.19 ^{ab}
	15°C		9.52 $\pm$ 5.54 ^{ab}	15°C		2.98 $\pm$ 2.98 ^{ab}	15°C		17.48 $\pm$ 5.61 ^{ab}
	20°C		4.46 $\pm$ 3.02 ^{ab}	20°C		4.29 $\pm$ 1.64 ^{ab}	20°C		4.16 $\pm$ 2.06 ^{ab}
	10°C	Farmed	4.51 $\pm$ 3.97 ^{ab}	10°C	Farmed	3.73 $\pm$ 1.83 ^{ab}	10°C	Farmed	1.44 $\pm$ 0.37 ^b

	15°C	3.97±0.68 ^{ab}	15°C	8.25±3.32 ^{ab}	15°C	9.21±5.67 ^{ab}
	20°C	18.63±12.76 ^a	20°C	16.11±8.19 ^{ab}	20°C	10.03±8.65 ^{ab}
<i>Ek</i>	10°C Natural	143.97±53.30 ^{ab}	10°C Natural	129.40±88.27 ^{ab}	10°C Natural	73.94±45.65 ^{ab}
	15°C	185.94±70.68 ^{ab}	15°C	111.63±58.52 ^{ab}	15°C	229.16±92.04 ^{ab}
	20°C	200.16±196.5 ^{ab}	20°C	111.39±48.50 ^{ab}	20°C	124.41±91.02 ^{ab}
	10°C Farmed	94.85±87.88 ^{ab}	10°C Farmed	66.76±32.14 ^{ab}	10°C Farmed	31.86±4.78 ^b
	15°C	93.56±21.17 ^{ab}	15°C	173.11±32.20 ^{ab}	15°C	110.38±70.53 ^{ab}
	20°C	442.97±306.60 ^a	20°C	233.80±108.15 ^{ab}	20°C	184.44±134.12 ^{ab}
<i>NPO_{max}</i>	10°C Natural	0.74±0.03 ^{ab}	10°C Natural	0.68±0.14 ^{ab}	10°C Natural	0.71±0.09 ^{ab}
	15°C	0.94±0.28 ^{ab}	15°C	0.42±0.50 ^a	15°C	1.03±0.27 ^{ab}
	20°C	0.64±0.14 ^{ab}	20°C	0.58±0.10 ^{ab}	20°C	0.55±0.20 ^a
	10°C Farmed	1.56±0.92 ^{ab}	10°C Farmed	0.66±0.13 ^{ab}	10°C Farmed	0.66±0.04 ^{ab}
	15°C	0.93±0.12 ^{ab}	15°C	1.16±0.23 ^b	15°C	0.99±0.30 ^{ab}
	20°C	0.69±0.09 ^{ab}	20°C	1.04±0.23 ^{ab}	20°C	0.86±0.10 ^{ab}