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

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

Sara Usandizaga

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

Carolina Camus

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

José Luis Kappes

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

Marie-Laure Guillemin

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

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

Alejandro H. Buschmann[2]

Centro i~mar and CeBiB, Universidad de Los Lagos, Camino Chinquihue 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

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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; *Ek*, 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 Guillemin et al. 2008) and is probably also present in

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

In Chile, intensive seaweed farming is limited to the domesticated red alga *Agarophyton chilensis* used mainly for agar extraction (Buschmann et al. 2017). Even if the domestication process of this species has begun only a few decades ago, the almost complete predominance of diploid individuals in farms demonstrate that farming practices had significantly modified life-history traits as compared to wild populations (Guillemin et al. 2008). Moreover, recent investigations have demonstrated that this red alga colonized the Chilean coast from New Zealand, likely at the end of the Last Glacial Maximum (Guillemin et al. 2014). The lower genetic diversity of the Chilean populations, when compared to the ones from New Zealand, is indeed consistent with a genetic bottleneck resulting from a transpacific range extension and was probably reinforced by the over-exploitation of natural Chilean populations during the 90´s (Guillemin et al. 2014). In Chile, active transport and exchange of inoculums of *A. chilensis* between coastal communities of fishermen for cultivation purposes have contributed to the artificial expansion of the species distribution. Nowadays, natural populations are distributed between 30°S to 45°S while farming extends further north, up to Antofagasta, 17°S (Bird et al. 1986, Guillemin et al. 2008). Human activities have also leaded to a loss of genotypic diversity in farmed populations that could be partially linked to involuntary selection of faster growing thalli, during the first steps of the domestication process (Guillemin et al. 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 µmol electron m^{-2} 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 Guillemin 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 (Guillemin 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 were *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 (Westermeier 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 µmol electron m^{-2} 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: $SGR= [\ln (Wf \cdot Wi^{-1})/ (tf-ti)] \times 100$; where Wi= initial fresh weight, Wf= 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, µmol electrons $\cdot$ $m^{-2} \cdot s^{-1}$) was determined after 20 s exposure in 12 increasing intensities of

177 PAR (up to 1500 µmol photon · m^{-2} · s^{-1}) provided by a blue light of the Junior PAM device
178 (Schreiber et al. 1995). ETR was calculated according to Schreiber et al. (1995) as follows:

179 $ETR = \Delta F/F'm \cdot E \cdot A \cdot F_{II}$ (µmol electrons · m^{-2} · s^{-1}) (1)

180 Where ΔF/F´m is the effective quantum yield, *E* is the incident PAR (photosynthetically
181 active radiation) irradiance expressed in µmol photons $m^{-2}s^{-1}$, *A* is the thallus absorptance
182 and F_{II} is the fraction of chlorophyll related to photosystem II (PSII, 400-700m), being 0.15
183 in red seaweed (Grzymski et al. 1997). As an estimator of photosynthetic efficiency, the
184 initial slope of ETR (α_{ETR}) and maximum ETR (ETR_{max}) were obtained from the tangential
185 function reported by Eilers and Peeters (1988) and the irradiance saturation (Ek_{ETR}) was
186 calculated from the intercept between these two parameters. Representing potential thalli
187 photoprotective mechanism, non-photochemical quenching (NPQ) was measured according
188 to Schreiber et al. (1995). The maximal non-photochemical quenching (NPQ_{max}) was
189 calculated from the tangential function of NPQ versus irradiance function according to
190 Eilers and Peeters (1988).

191 *Statistical treatment*

192 All analyses were performed in R (3.2.4 version) (Cayuela 2011). Assumptions of
193 homogeneity of variances and normal distribution were tested using Levene`s test and
194 Shapiro-Wilk, respectively. When non-normal residuals and heteroscedasticity were
195 detected, the data were transformed using logarithm (for *Ek* and SGR) or Box-cox (for
196 F_v/F_m). The experimental design fitted a three-way ANOVA with treatment (temperature:
197 10°C, 15°C and 20°C), site of origin (Concepción, Maullín and Ancud) and population type

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

200 **Results**

201 Significant differences were detected in *Agarophyton chilensis* specific growth rate (SGR)
202 after 30 days of experimentation between temperature treatments ($F_{(2, 72)} = 15.46$; $P <$
203 0.0001), between population types ($F_{(1, 72)} = 4.93$; $P= 0.03$) and a significant interaction was
204 also detected between population type and site of origin ($F_{(2, 72)} = 5.00$; $P= 0.009$). In
205 Maullín and Ancud, no significant differences between temperature treatments were
206 detected for farmed thalli (Fig.2C and E), while farmed thalli from Concepción showed a
207 slightly but significantly lower SGR at 20°C than at 15°C (7.26 ± 1.48 and 9.45 ± 1.92 g ·
208 day^{-1}, respectively; Fig.2A). In addition, differences among Concepción farmed and natural
209 populations were observed (Tukey test; $P<0.05$, see Figure 2A and B). In contrast, SGR
210 was significantly higher at 15°C for thalli sampled in natural *A. chilensis* stands, whatever
211 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
213 did not decrease significantly (Tukey test; $P<0.05$) at the lower (10 ^{o}C) and the higher (20
214 ^{o}C) temperature tested for Maullin and Ancud populations (Fig. 2 C, D, E and F).

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

220 ETR_{max} and *Ek* were significantly higher for thalli sampled in the farmed population of
221 Concepción and grown at 20°C than for thalli sampled in the farmed population of Ancud
222 and grown at 10°C (18.63 ± 12.76 and 1.44 ± 0.37 μmol electrons · m^{-2} · s^{-1}, 442.97 ±
223 306.60 and 31.86 ± 4.78 μmol photons · m^{-2} · s^{-1}, for ETR_{max} and *Ek*, respectively; Tukey's
224 HSD tests; $p < 0.05$; Table 2). Significant interactions between temperature treatment,
225 population type and site of origin were observed for F_v/F_m and NPQ_{max} (Table 1). In
226 Maullín at 15°C, values of NPQ_{max} were significantly higher for thalli sampled in the
227 farmed bed than the natural population (Tukey's HSD tests; $p < 0.05$; Table 2).

228 **Discussion**

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

240 Regardless of the origin of the natural population under study, our experiment shows that
241 thalli specific growth rate (SGR) was higher at 15°C than at lower (i.e., 10°C) or higher
242 (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-

266 purpose genotypes, able to grow in highly stressful and/or variable environments. General-
267 purpose-genotypes are sometime also referred as 'Jack-of-all-trades, master of none' since
268 they are described as versatile genotypes that are able to perform adequately across a range
269 of environments but are not superior in any of them. These general-purpose-genotypes can
270 confer a species or population a broad tolerance to environmental changes and are often
271 associated with species invasion (Baker 1974, Richards et al. 2006). In Chile, farms have
272 been developed using material growing embedded in muddy estuaries and sandy bays.
273 These habitats are typically highly heterogeneous and present strong seasonal variations in
274 temperature and salinity (Westermeier et al. 1993, Buschmann et al. 1995). It have been
275 demonstrated that intraspecific competition for resources utilization in clonal individuals
276 living in habitats characterized by fluctuating environmental conditions could lead to
277 positive selection of general-purpose-genotypes (Arnaud-Haond et al. 2012).
278 In benthic algae, temperature variations affect photosynthetic metabolism (Davison 1991)
279 determining, for example, seasonal distribution (De Nicola 1996). However, habitats
280 characterized by strong spatial and temporal variations of abiotic factors, request constant
281 adjustment of photosynthetic processes in species populating them (Ensminger et al. 2001).
282 In our study, the highest values of ETR_{max} and *Ek* were observed in the *A*. *chilensis* farmed
283 population from Concepción grown at 20°C. These results suggest that the effect of high
284 temperatures on photosynthetic metabolism of this farmed population could be mild.
285 Driven by seasonal changes in river discharge, precipitation, and coastal upwelling, high
286 variability in abiotic conditions (e.g., temperature, salinity and turbidity; Saldías et al.
287 2016) has been observed in the river mouths were *A*. *chilensis* thalli are planted in
288 Concepción. These characteristics could be associated to distinctive heat susceptibility of
289 the photosynthetic metabolism of the *A*. *chilensis* thalli growing in Concepción farms. In

290 our experiment, the response of the maximum quantum yield (F_v/F_m) to temperature was
291 quite variable. The lowest value of F_v/F_m (indicating photoinhibition) was observed at
292 intermediate temperature (15°C) in the farm of Maullín. In plants, it is well known that
293 limitation of electron transport that reduces the ability of plants to use light result in an
294 excess light energy that may cause photoinhibition due to damage to the PSII apparatus
295 (Moll and Steinback 1986). However, short-term response of photosynthesis to temperature
296 cannot easily be used to infer the long-term response of algal growth (Wienke and Dieck
297 1989). Indeed, despite the possible signal of photoinhibition detected at 15°C, no limited
298 growth could be observed in these *Agarophyton chilensis* thalli.
299 Populations of *Agarophyton chilensis* from different sites in Chile have been reported to
300 present ecological differences, potentially linked to local adaptation in response to specific
301 abiotic and biotic environmental characteristics (Santelices and Ugarte 1990, Usandizaga et
302 al. 2018). The success of *Agarophyton* farming depends in part on the origin of the initial
303 inoculum since differences in thallus morphology, agar yield and gel strength and
304 susceptibility to epiphytes exist among regions and populations. Indeed, it have been
305 suggested that random transplantation between distinct habitats could lead to cultivation
306 failure (Santelices and Ugarte 1990). However, in our experiment, even if we included
307 sampling sites located more than 700 km apart, no major effect of the site of origin were
308 detected on growth or photosynthesis. A possible explanation for this discrepancy is that
309 continuous transplantations and exchanges during the last decades have leaded to the
310 homogenization of the genetic diversity among the whole Chilean coast. However, this
311 hypothesis is not in accordance with population genetic studies showing the presence of
312 clear genetic divergence between regions in Chile (Guillemin et al. 2008 and 2014). Studies
313 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.

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341

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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 (±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

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

Sara Usandizaga

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

Carolina Camus

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

José Luis Kappes

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

Marie-Laure Guillemin

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

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

Alejandro H. Buschmann[2]

Centro i~mar and CeBiB, Universidad de Los Lagos, Camino Chinquihue 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; *Ek*, 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 Guillemin et al. 2008) and is probably also present in

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

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

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

Considering the possible consequences of unconscious selection (as defined in Zohary 2004) and domestication on ~~*G*~~*Agarophyton*~~.~~ *chilensis*, we propose that natural populations will be less sensitive to temperature variations than farmed populations. Genotypic diversity has been shown to be higher in natural than farmed *A. chilensis* Chilean populations and we propose that the farms will show less effective mechanisms of acclimation (e.g., enhancing of photosynthetic performance) than the one observed in natural populations when confronted with temperature considered as high in their natural environment (i.e., water of the southern coast of Chile do not generally reach 20°C; Westermeier et al. 1993). The aim of the present study was to assess the effect of temperature on growth and photosynthetic responses of both natural and farmed stands of *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 µmol electron m^{-2} 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 Guillemin 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 (Guillemin 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 were *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 (Westermeier 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 µmol electron m^{-2} 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: $SGR = [\ln (Wf \cdot Wi^{-1})/ (tf\text{-}ti)] \times 100$; where Wi= initial fresh weight, Wf= 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, µmol electrons $\cdot$ m^{-2} $\cdot$ s^{-1}) was determined after 20 s exposure in 12 increasing intensities of

177 PAR (up to 1500 µmol photon · m^{-2} · s^{-1}) provided by a blue light of the Junior PAM device
178 (Schreiber et al. 1995). ETR was calculated according to Schreiber et al. (1995) as follows:

179 $ETR = \Delta F/F'm \cdot E \cdot A \cdot F_{II}$ (µmol electrons · m^{-2} · s^{-1}) (1)

180 Where ΔF/F´m is the effective quantum yield, *E* is the incident PAR (photosynthetically
181 active radiation) irradiance expressed in µmol photons $m^{-2}s^{-1}$, *A* is the thallus absorptance
182 and F_{II} is the fraction of chlorophyll related to photosystem II (PSII, 400-700m), being 0.15
183 in red seaweed (Grzymski et al. 1997). As an estimator of photosynthetic efficiency, the
184 initial slope of ETR (α_{ETR}) and maximum ETR (ETR_{max}) were obtained from the tangential
185 function reported by Eilers and Peeters (1988) and the irradiance saturation (Ek_{ETR}) was
186 calculated from the intercept between these two parameters. Representing potential thalli
187 photoprotective mechanism, non-photochemical quenching (NPQ) was measured according
188 to Schreiber et al. (1995). The maximal non-photochemical quenching (NPQ_{max}) was
189 calculated from the tangential function of NPQ versus irradiance function according to
190 Eilers and Peeters (1988).

191 *Statistical treatment*

192 All analyses were performed in R (3.2.4 version) (Cayuela 2011). Assumptions of
193 homogeneity of variances and normal distribution were tested using Levene`s test and
194 Shapiro-Wilk, respectively. When non-normal residuals and heteroscedasticity were
195 detected, the data were transformed using logarithm (for *Ek* and SGR) or Box-cox (for
196 F_v/F_m). The experimental design fitted a three-way ANOVA with treatment (temperature:
197 10°C, 15°C and 20°C), site of origin (Concepción, Maullín and Ancud) and population type

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

200 **Results**

201 Significant differences were detected in *Agarophyton~~.~~ chilensis* specific growth rate (SGR)
202 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
203 also detected between population type and site of origin ($F_{(2, 72)} = 5.00$; $P = 0.009$). In
204 Maullín and Ancud, no significant differences between temperature treatments were
205 detected for farmed thalli (Fig.2C and E), while farmed thalli from Concepción showed a
206 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
207 populations were observed (Tukey test; $P<0.05$, see Figure 2A and B). In contrast, SGR
208 was significantly higher at 15°C for thalli sampled in natural *A*. *chilensis* stands, whatever
209 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
210 did not decrease significantly (Tukey test; $P<0.05$) at the lower (10 ºC) and the higher (20
211 ºC) temperature tested for Maullin and Ancud populations (Fig. 2 C, D, E and F).
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215 Regarding the photosynthetic responses, significant effect of temperature treatments were
216 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 (*Ek*~~; $F_{(2, 35)} = 6.97$; $P= 0.04$~~) and the maximal non-
217
218
219 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 ± 0.37 µmol electrons · m^{-2} · s^{-1}, 442.97 ± 306.60 and 31.86 ± 4.78 µmol photons · m^{-2} · 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)

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 (Westermeier 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 *Ek* 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 were *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.

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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 (±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

Map and photographs

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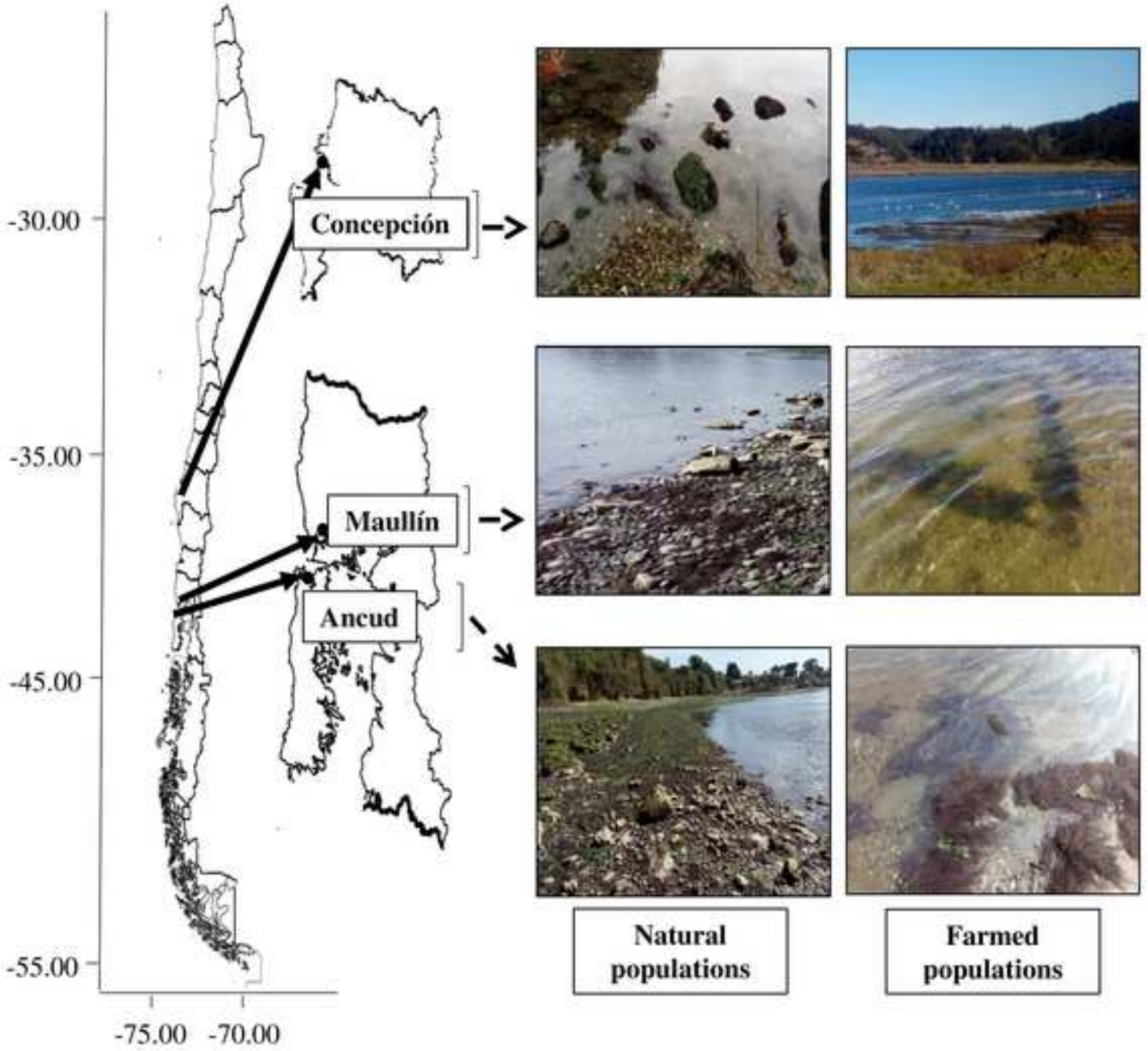

growth results

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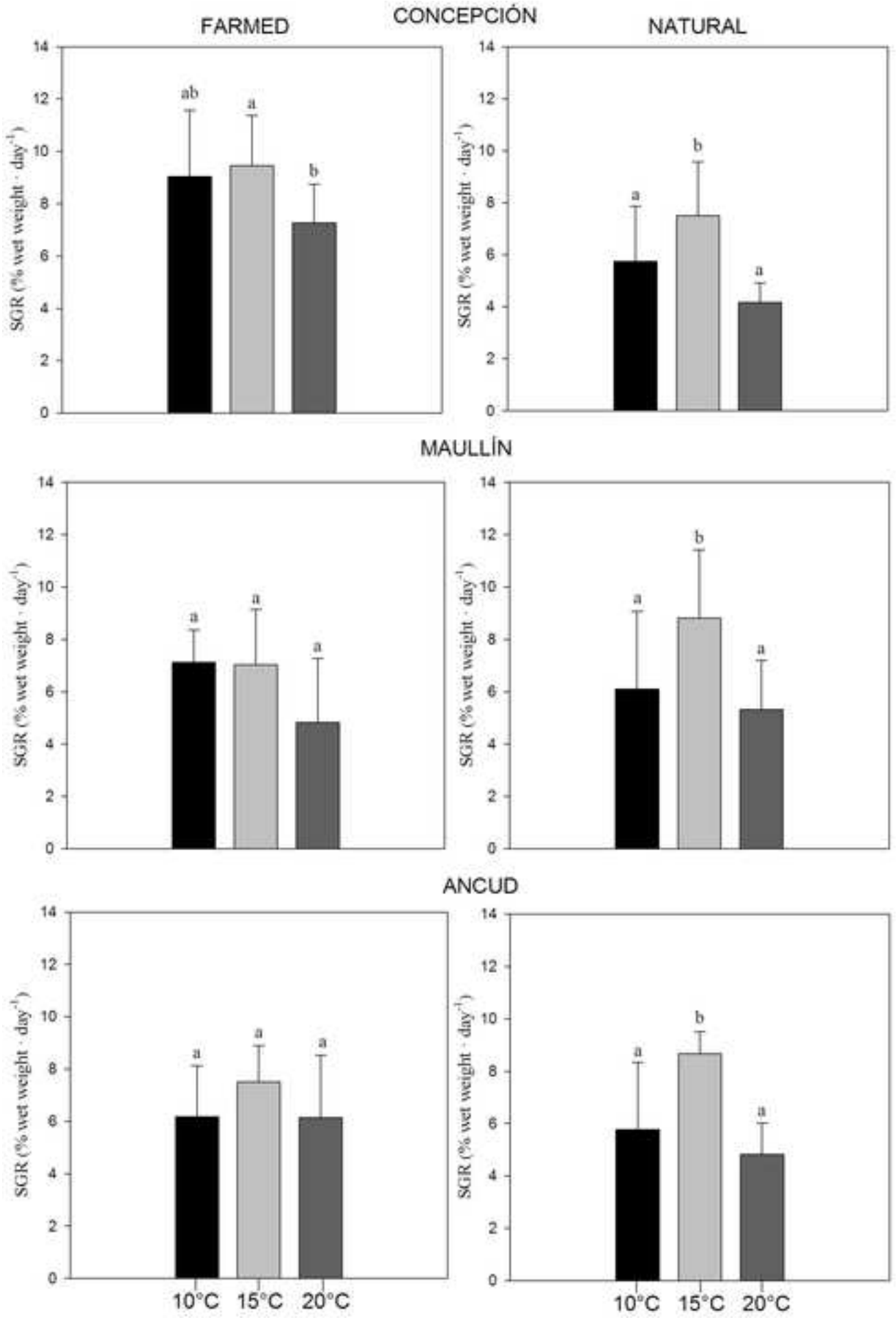

Table 1

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Table 1. Results of ANOVA analyses on of the specific growth rate (SGR) and photosynthetic parameters for *Agarophyton 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	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
Ek	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 ± S.E.~~D~~ (n = 5). Distinct uppercase letters denote significant differences after Tukey test.

	CONCEPCIÓN			*MAULLÍN*			*ANCUD*		
	T	**P**	**Means±S.E.**	**T**	**P**	**Means±S.E.**	**T**	**P**	**Means±S.E.**
F_v/F_m	10°C	Natural	0.60 ± 0.01^{ab}	10°C	Natural	0.64 ± 0.03^{ac}	10°C	Natural	0.66 ± 0.01^{ac}
	15°C		0.64 ± 0.02^{abc}	15°C		0.37 ± 0.36^{b}	15°C		0.64 ± 0.02^{abc}
	20°C		0.66 ± 0.02^{ac}	20°C		0.66 ± 0.01^{ac}	20°C		0.66 ± 0.01^{abc}
	10°C	Farmed	0.68 ± 0.02^{c}	10°C	Farmed	0.69 ± 0.00^{c}	10°C	Farmed	0.66 ± 0.01^{ac}
	15°C		0.61 ± 0.02^{ab}	15°C		0.61 ± 0.02^{ab}	15°C		0.64 ± 0.02^{abc}
	20°C		0.64 ± 0.03^{abc}	20°C		0.63 ± 0.01^{abc}	20°C		0.65 ± 0.02^{abc}
ETR_{max}	10°C	Natural	5.33 ± 0.39^{ab}	10°C	Natural	5.94 ± 3.28^{ab}	10°C	Natural	3.06 ± 1.19^{ab}
	15°C		9.52 ± 5.54^{ab}	15°C		2.98 ± 2.98^{ab}	15°C		17.48 ± 5.61^{ab}
	20°C		4.46 ± 3.02^{ab}	20°C		4.29 ± 1.64^{ab}	20°C		4.16 ± 2.06^{ab}
	10°C	Farmed	4.51 ± 3.97^{ab}	10°C	Farmed	3.73 ± 1.83^{ab}	10°C	Farmed	1.44 ± 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}
Ek	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}
NPQ_{max}	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}