

# Growth responses to temperature, salinity and nutrient variations, and biomass variation and phenology of *Ahnfeltia plicata* (Rhodophyta, Ahnfeltiales): a commercially interesting agarophyte from the Magellanic Region, Chile

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**Abstract** *Ahnfeltia plicata* (Hudson) E.M. Fries (Rhodophyta, Ahnfeltiales) is one of the most commercially important agarophytes in the world for its production of agar that is high quality and low in sulfate content. In the Magellanic Region, *A. plicata* forms extensive beds with high biomass production, which could be commercially exploited for agar production. The purposes of this study were to determine the optimal conditions of temperature, salinity, and culture medium; to evaluate the effects of different types and concentrations of auxins and cytokinins on growth of red and yellow gametophytes; and to provide background information on ecological parameters of natural population of *A. plicata*. Temperatures of 5, 8, 15, and 23 °C were tested, and the interaction of salinity of 25 and 35 psu with Provasoli enriched medium in half (PES/2) and quarter strength (PES/4), and with von Stosch enriched medium in half (VSES/2) and quarter strength (VSES/4) was also conducted. Concentrations of 5.0 and 50.0 µM of two auxins (indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D)), and two cytokinins (isopentenyladenine (iP) and benzylaminopurine) were added to VSES medium and gelled with 0.5 % agar. Each treatment was tested with three replicates. Red gametophytes of *A. plicata* tolerate a range of temperature

variation, from 5 to 23 °C, and the optimum temperature for growth was 15 °C. The highest growth rate was observed in salinity of 35 psu with half strength of von Stosch culture medium. Red and yellow gametophytes showed different responses to plant growth regulators, and red gametophytes were more sensitive than yellow ones to the addition of IAA and high concentration of iP. However, growth of red gametophytes of *A. plicata* was stimulated by 2,4-D. The differential sensitivity of red and yellow gametophytes to plant growth regulators suggests the need to test other types and concentrations of auxins and cytokinins.

**Keywords** Ahnfeltiales · Auxins · Cytokinins · Phenology · Salinity · Temperature

## Introduction

*Ahnfeltia plicata* (Hudson) E.M. Fries (Rhodophyta, Ahnfeltiales) is considered one of the most commercially important agarophytes in the world (Maggs and Pueschel 1989) producing a high quality and low sulfate agar (Chapman and Chapman 1980). Some species of *Ahnfeltia*, the only genus recognized in this order, are an important source of phycocolloids (Sukhoverkhov et al. 2000) and are of ecological importance as they form extensive beds, which serve as food and habitat for benthic fauna (Pavlyuk et al. 2008; Skriptsova and Nabivailo 2009; Milstein and Saunders 2012). It is harvested mainly on the Russian coast of the White Sea (Maggs et al. 1989). The distribution of *A. plicata* is conspicuously disjunct, with circumpolar distribution in the northern and southern hemispheres, occurring in the North

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Atlantic Ocean, in the northern North Pacific and southern South Atlantic and South Pacific Oceans (Maggs et al. 1989; Milstein and Saunders 2012).

The morphology of *A. plicata* gametophytes is composed of erect axes, with 3 to 21 cm long, which arise in tufts from crustose holdfast (1 cm diameter). Branching is generally irregular and variable, with some axes forming dichotomies or irregularly branches. Male gametophytes form spermatangial sori in mature axes and are absent in apical and basal regions of the axes. Similarly, female sori are formed in mature axes and usually formed along one side of the axis (Maggs and Pueschel 1989).

The life history of *A. plicata* from species found in Canada is heteromorphic, and it is characterized by the presence of an erect thallus of gametophytes and crustose tetrasporophytes, which grow very closely attached to hard substrata (Maggs and Pueschel 1989; Milstein and Saunders 2012). However, the life history of *A. plicata* from the South Atlantic and South Pacific Oceans remains unknown.

In Chile, the occurrence of *A. plicata* was reported in Tierra del Fuego (Wiencke and Clayton 2002), Magellan Region, and Diego Ramírez Islands (Mansilla and Navarro 2003). In the Magellan Region, *A. plicata* grows on rocky substrates from the intertidal zone to 20 m depth, in sheltered or wave-exposed sites. Along the exposed coast of Riesco Island (Seno Skyring, Magellan Region), *A. plicata* forms extensive beds producing a biomass of 3 kg m<sup>-2</sup> (fresh weight), with a 20 % yield in dry weight. In relation to biomass, larger abundance values are observed during autumn whereas lower values are observed in winter. The highest percentages of fertile plants occur during the spring while the lowest in autumn. Phenotypes with pigment variations are easily detectable and they can be used as markers for the isolation of highly productive strains (Zhang and Van der Meer 1987; Niwa et al. 2002) or as markers in physiological and biochemical studies of pigment composition, life history, photosynthetic processes, and polysaccharides composition and growth (Kursar et al. 1983a, b; Ramus and Van der Meer 1983; Costa and Plastino 2001; Guimarães et al. 2003; Yokoya et al. 2003).

The ranges of tolerance to salinity in agarophytes species of commercial interest can be extremely variable and it is one of the important factors influencing its growth (Kaliaperumal et al. 1993). The optimum growth of some species of red algae depends on its reproductive phase and could limit their distribution (Mansilla et al. 2004; Ramlov et al. 2012). There are few studies on responses to different salinities; nevertheless, there are several reports relating to photoperiod, light intensity, and temperature (Avila et al. 1985; Müller and Lüthe 1981; Müller and Meel 1982; Stolpe et al. 1991).

Temperature is a parameter that causes similar behaviors in cold temperate zones in both hemispheres (Sundene 1962; Munda and Liining 1977; Lüning 1984; Lüning and Freshwater 1988). van den Hoek (1982a, b) and Breeman

(1988) showed that the geographical distribution of macroalgae depends on tolerance to temperature, requirements for growth and reproduction through the different stages of the life cycle. In the context, it is important to study tolerance levels to changes in temperature, as they could indicate adaptative survival strategies to large disturbances (Wiencke and Dieck 1990). Santelices and Marquet (1998) established that Antarctic affinities species can reach several Pacific temperate latitudes throughout the South America due to their optimum and tolerance limits that allow them to survive to temperature variations.

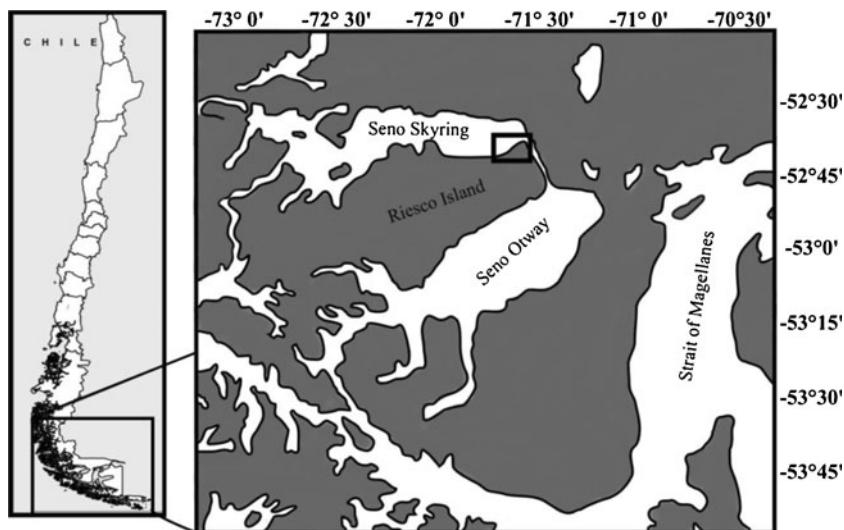
Extracts of *A. plicata* collected during the spring, summer, and autumn, from Riesco Island, showed the presence of agarans (Ortiz 2011). Chemical analysis of agarans obtained during different seasons showed that the content of galactose and 3, 6-anhydro-galactose is in a 1:1 ratio and elemental analysis showed that the agarans does not present sulfur content indicating that the neutral agaran presents the structure of agarose (Ortiz 2011). This agar is used as gelling and thickening agents in food industries and stabilizing agent in cosmetic and pharmaceutical industries (Chapman and Chapman 1980). However, despite the commercial importance of *A. plicata*, its physiological responses are poorly known in the world, especially for the species found in South Atlantic and South Pacific Oceans.

The purposes of this study were to determine the optimal conditions of temperature, salinity, and culture medium; to evaluate the effects of different types and concentrations of auxins and cytokinins on growth of *A. plicata* gametophytes; and provide background information on ecological parameters of natural populations in Seno Skyring.

## Materials and methods

*Seasonal variation on biomass and phenology of natural populations of A. plicata* Destructive sampling was used to estimate the seasonal variation of biomass (g cm<sup>-2</sup>) in a natural meadow in Seno Skyring, Riesco Island (52°38' 59.7"S 71°29'44"W, Fig. 1). In a meadow of approximately 2.5 km long and 10 m wide, five sampling sites were selected. Each site was separated by 0.5 km and included the whole extension of the meadow where randomly six quadrats (25 × 25 cm) were sampled and all the fronds removed, obtaining a total of 30 quadrants in each season of the year. The fronds collected from each quadrat were deposited in bags, labeled, and stored in containers with appropriate temperature and humidity conditions to be transported to the laboratory. Each sample was analyzed in its set, separating every frond according to its reproductive phase. The wet biomass was obtained weighing all the fronds per quadrat immediately after their collection.

**Fig. 1** Location of *Ahmfeltia plicata* natural population, Skyring Sound in Riesco Island, Chile. The square show de sampling site



**Laboratory experiments** Fertile gametophytes of *A. plicata* were collected in Seno Skyring (52°38'59.7"S 71°29'44"W), Magellan Region, Chile. Experiments of temperature, salinity, and culture medium were performed with red specimens collected in October 2010. Experiments of plant growth regulators were performed with specimens collected in March 2011, which presented red and yellow phenotypes. The bottom water of Seno Skyring is characterized by salinities of 19–19.5 psu, low oxygen contents of 6–7 mg L<sup>-1</sup> and low temperatures of around 6 °C which corresponds to the average annual temperature at sea level of this area (Kylian et al. 2007).

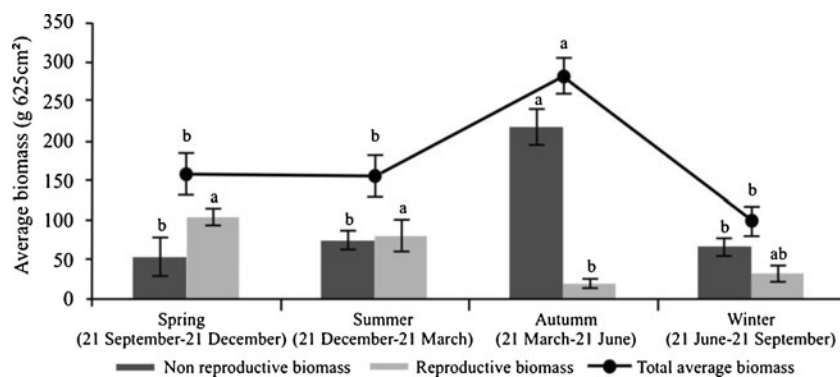
Apical segments (20 mm long) of gametophytes of the two collecting periods were cultured in seawater enriched with half strength of von Stosch's solution (VSES/2 medium) prepared as described by Oliveira et al. (1995) and modified with reduction of 50 % in the vitamin concentrations (Yokoya 2000).

**Experiments of temperature and the interaction between salinity and culture medium** Variations of temperature (5, 8, 15, and 23 °C) and the interaction of two salinities (25 and 35 psu) and two culture media [Provasoli enriched medium in a half (PES/2) and a quarter strength (PES/4), and von Stosch enriched medium in a half (VSES/2) and a quarter strength (VSES/4)] were tested. Other experimental conditions were light/dark cycle of 16:8 h, photon flux density of 6±1 μmol photons m<sup>-2</sup> s<sup>-1</sup>, and salinity of 35 psu (temperature experiment) and 5 °C (salinity/culture medium experiment). Each treatment was tested with three replicates ( $n=3$ ) of five apical segments (5 mm long). Variation of fresh biomass (mg) was recorded weekly in the same intervals of medium renewal. The growth rate was calculated as  $[\ln(B_f B_o^{-1})t^{-1}]$ , where  $B_o$  is the initial fresh biomass,  $B_f$  is the fresh biomass after  $t$  days, and  $t$  is the number of days (Yokoya et al. 2007).

**Experiments with plant growth regulators** Apical segments (20 mm long) isolated from red and yellow gametophytes were cultured during 1 week in VSES/2 medium under temperature of 8±1 °C, and the other experimental conditions were the same as described to temperature experiment.

Apical segments (10 mm long) were cut from the red and yellow specimens cultured as above described and sterilized through the following protocol: (a) segments were washed for 10 s in a solution of sterile seawater with 0.5 % sodium hypochlorite and 200 μL L<sup>-1</sup> of detergent, and (b) washed several times in sterile seawater to remove the previous solution. These explants were inoculated into 30 mL of sterilized full-strength VSES solid medium supplemented with plant growth regulators PGR. Concentrations of 5.0 and 50.0 μM of two auxins [indole-3-acetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D)] and two cytokinins [isopentenyladenine (iP), and benzylaminopurine] were added to the full-strength VSES medium, and gelled with 0.5 % agar. Each treatment was tested with three replicates. After 4 weeks in solid medium, explants were cultured in a liquid medium with the same experimental conditions. Fresh biomass (mg) was recorded weekly in the same intervals of medium renewal.

**Statistical analysis** The data for the seasonal variation of biomass from natural populations of *A. plicata* were analyzed with the nonparametric Kruskal–Wallis ANOVA test. The data of the laboratorial experiments were analyzed with a one-way analysis of variance (ANOVA). Tukey's multiple comparison tests were conducted to distinguish significantly different results ( $p<0.05$ ) following the ANOVA test (Zar 1999). Statistical tests of seasonal variation and laboratorial results were performed using Statistica, version 6.0 software.



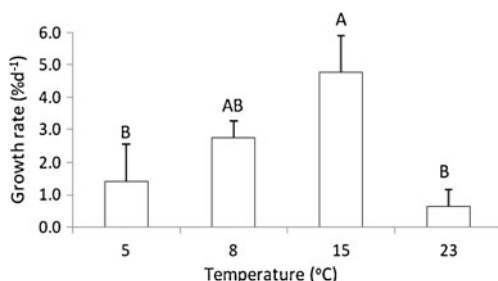
**Fig. 2** Seasonal variation of the total average biomass, the non-reproductive biomass, and reproductive biomass of *A. plicata* in a natural population in Skyring Sound, Magellan Region, Chile. Different letters

indicate statistically significant differences. Values of bars marked by the same letter are not significantly different according to the Kruskal–Wallis ANOVA test ( $p=0.05$ )

**Results**

*Seasonal variation biomass and phenology of natural populations of A. plicata* The average biomass ( $\text{g cm}^{-2}$ ) presented a well-marked fluctuation between summer (January) and autumn (May), and autumn and winter (July), showing a significant increase in biomass in autumn ( $281.64 \pm 23.21 \text{ g cm}^{-2}$ ) ( $p=0.01$ ), and a significant biomass reduction during winter season ( $98.7 \pm 17.32 \text{ g cm}^{-2}$ ) ( $p=0.0001$ ) (Fig. 2).

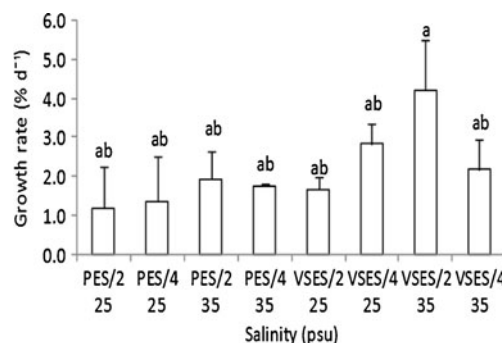
Phenologically, *A. plicata* had the highest records of fertile average biomass during the spring and summer periods:  $104.13 \pm 9.78$  and  $80.25 \pm 20.17 \text{ g cm}^{-2}$ , respectively), where more than 50 % corresponded to reproductive plants. The average biomass of fertile plants tended to decrease during winter, finding the lowest average biomass in autumn ( $19.93 \pm 6.06 \text{ g cm}^{-2}$ ). Significant differences in autumn were only detected in relation to other seasonal periods ( $p=0.0001$ ) (Fig. 2). In general, the biomass averages of fertile and infertile plants were similar to almost all seasonal periods, excepting for the autumn, when there was a higher increase of average biomass of fertile plants in relation to reproductive plants (Fig. 2).



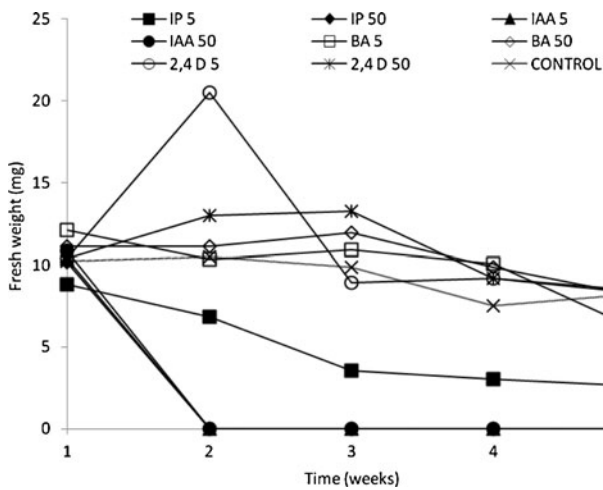
**Fig. 3** Growth rates of (fresh biomass variation) *A. plicata* gametophytes cultured in different temperatures. Each data point is the mean of three replicates ( $n=3 \pm$  standard deviation). Values of bars marked by the same letter are not significantly different according to ANOVA and Tukey's multiple comparison test ( $p=0.05$ )

*Effects of temperature and the interaction of salinity and culture medium* Gametophytes tolerated a wide range of temperature variation, from 5 to 23 °C, and the lowest growth rates were observed in the extreme of temperatures (1.4 and 0.6 %  $\text{day}^{-1}$  for 5 and 23 °C, respectively), and the highest growth rate (4.8 %  $\text{day}^{-1}$ ) was observed at 15 °C (Fig. 3). The optimum combination of salinity and culture medium was 35 psu with VSES/2 medium, and the maximum growth rate was 4.4 %  $\text{day}^{-1}$ , and the other treatments were not different significantly (Fig. 4).

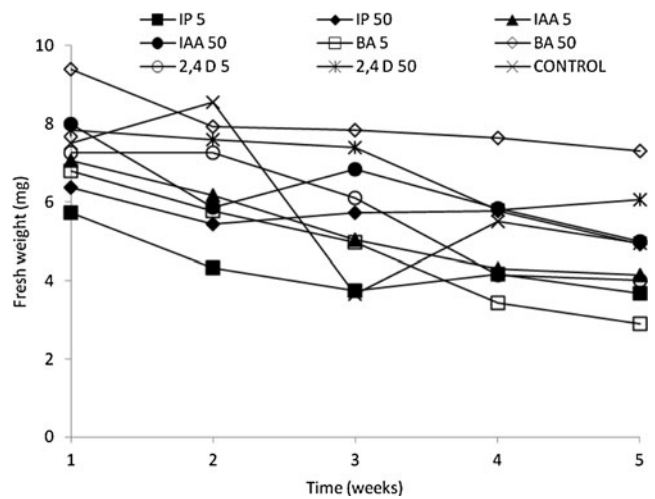
*Effects of plant growth regulators* Growth of red gametophytes of *A. plicata* was stimulated by 2,4-D at the concentrations of 5.0 and 50.0  $\mu\text{M}$  during, respectively, the second and fourth weeks; afterwards, the growth decreased (Fig. 5). Red gametophytes did not tolerate treatments with the auxin IAA (in concentrations of 5.0 and 50.0  $\mu\text{M}$ ) and with high concentration of the iP. On the other hand, growth of yellow gametophytes of *A. plicata* was inhibited by all treatments



**Fig. 4** Growth rates of (fresh biomass variation) *A. plicata* gametophytes cultured in two salinities (25 and 35 psu) combined with Provasoli enriched medium in half (PES/2) and quarter strength (PES/4), and with von Stosch enriched medium in half (VSES/2) and quarter strength (VSES/4). Each data point is the mean of three replicates ( $n=3 \pm$  standard deviation). Values of bars marked by the same letter are not significantly different according to ANOVA and Tukey's multiple comparison test ( $p=0.05$ )



**Fig. 5** Growth of red gametophytes of *A. plicata* cultured with 5.0 and 50.0  $\mu\text{M}$  of auxins (indole-3-acetic acid=*IAA*, 2,4-dichlorophenoxyacetic acid=*2,4-D*) or cytokinins (isopentenyladenine=*iP*, and benzylaminopurine=*BA*) which were added to VSES medium. Each data point is the mean of three replicates ( $n=3\pm$ standard deviation)



**Fig. 6** Growth of yellow gametophytes of *A. plicata* cultured with 5.0 and 50.0  $\mu\text{M}$  of auxins (indole-3-acetic acid=*IAA*, 2,4-dichlorophenoxyacetic acid=*2,4-D*) or cytokinins (isopentenyladenine=*iP*, and benzylaminopurine=*BA*) which were added to VSES medium. Each data point is the mean of three replicates ( $n=3\pm$ standard deviation)

with auxins and cytokinins (Fig. 6), but these phytohormones were not lethal. Therefore, red and yellow gametophytes showed different responses to plant growth regulators, and red gametophytes were more sensitive than yellow ones to the addition of IAA and high concentration of *iP*.

## Discussion

**Seasonal variation on biomass and phenology of natural populations of *A. plicata*** During autumn (21 March–21 June), *A. plicata* showed a marked fluctuation in biomass average, with the highest average biomass in autumn and the lowest during winter (21 June–21 September). Despite the fluctuation of the average biomass of *A. plicata* this species can be found throughout the four seasons of the year. These results have already been reported for other natural meadows of *A. plicata* of the Northern Hemisphere (Hehre and Mathieson 1970; Davies and Wilce 1987; Airoldi 2003), and these authors also considered this species as a perennial algae. Airoldi (2003) mention that the growth “peak” of natural meadows of *A. plicata* in the Northern Hemisphere are related to the seasonal fluctuations of sedimentation on rocky coasts. The results on the average seasonal biomass differ from other studies with other species of the genera *Ahnfeltia*, as in the case of *Ahnfeltia tobuchiensis* (Kanno & Matsubara) Makienko, which displays the highest values of biomass in summer (21 June–21 September Northern Hemisphere) and presents a decline in biomass in autumn (Cherbadgy and Popova 1998). These results are the first records on the dynamic population of *A. plicata* and represent important findings for future measurements of sustainable uses of natural

populations in the sub-Antarctic ecoregion in Southern Hemisphere.

**Effects of temperature and the interaction of salinity and culture medium** The highest growth rates for *A. plicata* observed in laboratory conditions were 15 °C and 35 psu. These results indicate that its ecological optima are different from its physiological optima since the temperature and salinity recorded at Seno Skyring are 6 °C and 19–19.5 psu (Kylian et al. 2007). Similar responses were observed in three red algae cultured in axenic conditions, and the optimum temperatures were 20–25 °C, and the water temperature in their habitats was up to 15 °C (Fries 1966). These results provide valuable information to obtain a higher biomass production of *A. plicata* by performing cultures at temperature and salinity that correspond to the optimum growth conditions.

**Effects of plant growth regulators** Growth of red gametophytes of *A. plicata* was stimulated by 2,4-D, and this stimulatory effect was also observed in apical and intercalary segments of *Gracilariopsis tenuifrons* (Bird & Oliveira) Fredericq & Hommersand (Yokoya 2000) and *Gracilaria vermiculophylla* (Ohmi) Papenfuss (Yokoya et al. 1999). The stimulatory effect of 2,4-D on seaweed growth could be related to its function on processes of cell division and elongation (Krikorian 1995).

The red and yellow gametophytes of *A. plicata* showed different responses to auxins and cytokinins, and similar results were also observed in phycoerythrin-deficient and wild strains of *Hypnea musciformis* (Wulfen in Jacqu.) J.V. Lamour. (Martins et al. 2008). These differences between color strains of *A. plicata* and *H. musciformis* may be explained by the biochemical differences between these

strains what could influence the cell sensitivity to the plant growth regulators (Davies 1995).

Based in our results, we could conclude that fertile gametophytes of *A. plicata* had the highest records of average biomass during the spring and summer periods; under laboratory conditions, it tolerates a wide range of temperature variation, and the optimum temperature and salinity/culture medium for growth were 15 °C and 35 psu with VSES/2 medium. The low tolerance of red gametophytes and the differential sensitivities between the red and yellow gametophytes of *A. plicata* to the exogenous plant growth regulators suggest the need to test other types and concentrations of auxins and cytokinins. This study contributes to the knowledge of the ecophysiology of *A. plicata*, which is important for improving the cultivation and sustainable use of this resource in the Magellan Region and the Chilean Antarctica.

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