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In vitro recruitment of *Ulva* sp. and *Enteromorpha* sp. on gametophytic and tetrasporophytic thalli of four populations of *Chondracanthus chamissoi* from Chile

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Abstract *Chondracanthus chamissoi* is a red alga which is acquiring an increasing economic importance in Chile and becoming a target species for cultivation. Because epiphytism is a major problem in the mariculture of seaweeds, the recruitment of *Ulva* sp. and *Enteromorpha* sp. on gametophytic (cystocarpic) and tetrasporophytic thalli of *C. chamissoi* was tested *in vitro* on four populations from different locations in Chile. For *Ulva* sp., the density, cover and length of the recruits varied between the reproductive phases of the host and among the studied populations of *C. chamissoi*. In most experiments, a larger number of epiphytic thalli was observed on gametophytic plants of *C. chamissoi*; populations from Calderilla and Lechagua had a higher resistance to epiphytism. For *Enteromorpha* sp., its ability to grow epiphytically on both phases of the host showed a greater variability, but was inferior to that of *Ulva* sp. The differential resistance to epiphytism in both reproductive phases and in plant origin indicates the possibility of obtaining selected strains of *C. chamissoi* with lower susceptibility to epiphytism that could be utilized in planning maricultural programs.

Key words Chile · *Chondracanthus chamissoi* · *Enteromorpha* · epiphytism · rhodophyta · *Ulva*

Introduction

Chondracanthus chamissoi (C. Agardh) Kützing is a benthic marine red alga that may reach up to 50 cm in length. It is found from the lower intertidal zone to depths of 15 m (Hoffmann and Santelices 1997), and is endemic to the region that extends from Paita, Peru (5°S) to Ancud, Chile (42°S) (Ramírez and Santelices 1991). This species has been commercialized for the extraction of carrageenan (Hoffmann and Santelices 1997). There is, however, a growing interest in the Asian market for acquisition of this product for direct human consumption. Although it attains attractive prices in world markets as a sea vegetable, quality standards are strict, requiring a clean product, free of epiphytes and impurities, and with specific color and texture. This is a constraint to exportation because natural populations of *C. chamissoi* present a broad morphological variation (Acleto 1986) and are subject to epiphytism, particularly by *Ulva* and *Enteromorpha* spp. (Vásquez and Vega 2001).

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Epiphytism has been considered one of the bigger problems in seaweed cultivation (Fletcher 1995; Oliveira et al. 2000; Ask and Azanza 2002; Lüning and Pang 2003). Massive monocultures of algae offer ideal conditions for the growth of epiphytes and propagation of diseases (Wheeler et al. 1981; Fletcher 1995; Lüning and Pang 2003). Epiphytes also produce a series of undesired effects in the production of the host depending on the extent of the contamination (Fletcher 1995). The more important problems with epiphytes have been attributed to: (1) competition for nutrients (Buschmann and Gómez 1993); (2) shading (Fletcher 1995); (3) increased weight and drag (Kuschel and Buschmann 1991; Buschmann and Gómez 1993); (4) the possible liberation by the epiphyte of growth inhibiting compounds that affect the host (Santelices and Varela 1993); and (5) a decrease in the reproductive output of the infected host (D'Antonio 1985).

Methods for controlling epiphytes have been described and utilized in cultures of *Gracilaria* (cf. Fletcher 1995). However, many of these methods can only be used in cultivation tanks and are more difficult to apply to open ocean cultivation.

The concern in seaweed mariculture with epiphytism has lead to special attention being directed to the selection of resistant strains and to knowledge of the strategies developed by the host to avoid epiphytism (e.g., Santelices and Ugarte 1990; Santelices 1992; Fletcher 1995; Ask and Azanza 2002). For example, the ability to produce compounds which deter the settlement and growth of epiphytic organisms has been described in some algae of commercial value (Santelices 1992; Jennings and Steinberg 1997; Harder et al. 2004; Hellio et al. 2004). On the other hand, it has been demonstrated that the capacity to resist an attack depends on the host susceptibility to epiphytes. For *Chondrus crispus*, it has been demonstrated that the presence of different types of carrageenan, in the different reproductive phases, confers a differential adaptation in recognizing an attack by epiphytic organisms (Bouarab et al. 1999, 2001).

In northern Chile, it has been demonstrated that *Chondracanthus chamisso*i shows reproductive (González and Meneses 1996), ecological (González et al. 1997), and physiological differences in both phases of its life cycle (Bulboa and Macchiavello 2001). Although there is no published record of differential resistance in *C. chamissoi* against epiphyte attack, we believe that this may occur in a manner similar to *C. crispus*.

Here, we evaluate the resistance to epiphytism of gametophytic and tetrasporophytic phases of *C. chamissoi* against the main epiphytes *Ulva* and *Enteromorpha* spp. We also tested the existence of possible differences against epiphytism among strains from different locations along the Chilean coast.

Materials and methods

Unialgal culture

Samples of 100 bearing cystocarps and 100 fronds bearing tetrasporanges were collected from each of the following localities: Calderilla, Herradura, Puerto Aldea, and Lechagua (Figure 1). Three 2-cm-long apical branches without reproductive structures were sectioned from each frond and carefully washed with sterilized sea water to remove debris and contaminating organisms. Totals of 300 tips from each phase were maintained in separate bottles (150 tips per bottle) with 2 l of sterile sea water enriched with half-strength von Stosch medium (Edwards 1970). The bottles were maintained at 15°C, 50 μmol photons m^{-2} s^{-1}, 12:12 h photoperiodl and constant aeration. At the start of the cultivationsl 6 ml of GeO_2 (3 μg L^{-1}) (Oliveira et al. 1995) and Potassium Penicillin (0.2 mg mL^{-1}) (Garcia-Jimenez et al. 1999) were added in order to control possible contamination by diatoms and inhibit cyanobacteria growth, respectively. The cultivation medium was renewed weekly. Tips were isolated into culture 3 months before the beginning of the experimental phase.

Experimental set-up

Species of *Ulva* and *Enteromorpha* were selected as epiphytes. Although we realize that these genera were recently synonymized (Hayden et al. 2003), we opted to maintain the traditional morphological concept and treat them separately in this study. These algae were collected in La Herradura Bay and transported to the laboratory. *Ulva* and *Enteromorpha* fronds were washed with abundant sterile sea water. Ten fronds of *Ulva* sp. and 10 g of *Enteromorpha* sp. were deposited in plastic receptacles with reduced light

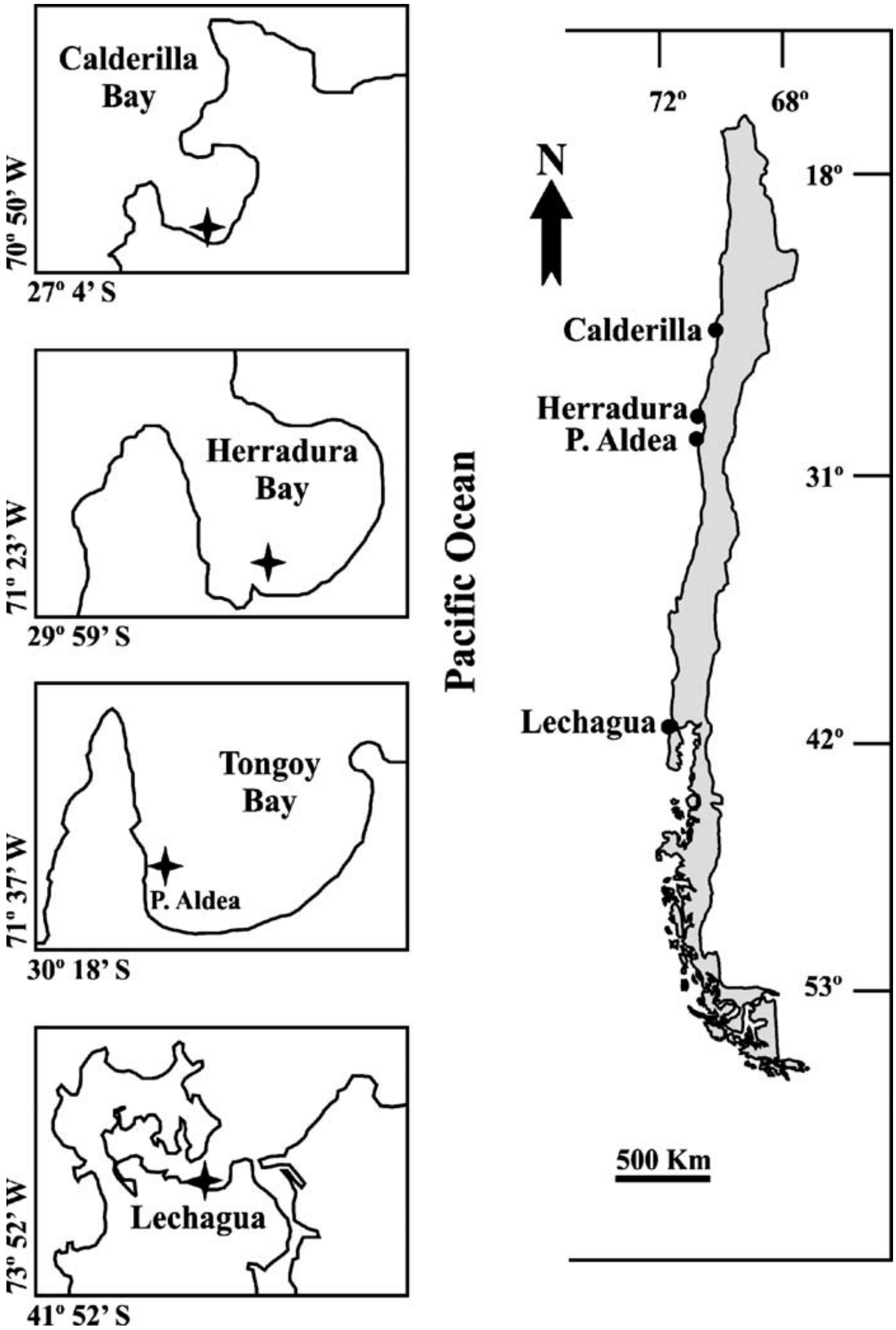

Figure 1 Geographic locations of the natural beds of *Chondrachanthus chamissoi* included in this study.

(10 μmol photons m^{-2} s^{-1}) at 15°C and without water for a period of 2 h and then immersed in 500 mL of sea water in order to stimulate the release of zooids. Aliquots were taken from the suspension of zooids and counted in a Neubauer hematocytometer. The concentration was adjusted to 5,000 zooids mL^{-1} by dilution with sterile seawater.

Five Petri dishes containing 60 mL of sterile medium and 3 cm long tips of *C. chamissoi* from each location and reproductive phase were inoculated with 10 mL of a suspension of *Ulva* sp. zooids. The same procedure was carried out for the inoculation of *Enteromorpha* sp. zooids. The control consisted of five Petri dishes for each epiphyte in which 10 mL of each of the zooids were added separately to dishes without fronds of *C. chamissoi*.

The experimental flasks were maintained at 15°C, 60 μmol photons m^{-2} s^{-1} and 12:12 h photoperiod. The cultivation medium was renewed weekly. Before beginning the aforementioned experiments, *C. chamissoi* thalli were cultivated under the same experimental conditions for 2 weeks to assure the absence of epiphytes.

Thirty days after epiphyte inoculation the *C. chamissoi* thalli were photographed with a digital camera (Canon PowerShot G3) in a stereoscopic microscope (40×). The photographs were analyzed with the program Image Pro Plus (Version 4.5) for: (1) number of epiphytes per mm^2; (2) length of epiphytic thalli in mm; and (3) pecentage coverage of *C. chamissoi* thalli by epiphyte for each of the localities studied.

The number, length and coverage data for both epiphytes were analyzed separately. The homocedasticity and normality of all of the results were checked, followed by a two-way ANOVA (location and reproductive phase) for each type of epiphyte. A Tukey test was utilized to test differences among groups.

Results

Ulva sp. and *Enteromorpha* sp. grew as epiphytes on *C. chamissoi* thalli, although the intensity of their growth varied between the life stages of the host and among locations of the populations studied.

Density

Figure 2a shows that the density of *Ulva* sp. was larger on the gametophytic than on the tetrasporophytic thalli of *C. chamissoi*. Differences were related to a first order interaction between the phases and the locations (Fc=4.2437; $P<0.05$), in which Calderilla, P. Aldea and Lechagua were significant ($P<0.05$). The control treatment presented higher density values than those registered on the tetrasporophytic thalli from the four locations, although these differences were significant only for Calderilla, P. Aldea and Lechagua ($P<0.05$).

For *Enteromorpha* sp., densities were smaller than the ones registered for *Ulva* sp. with maximum values of 59±17 and 51±25 (microthalli mm^{-2}) for gametophytic and tetrasporophytic thalli respectively (Figure 2b). Differences between locations were not significant (Fc=1.7740, $P>0.05$).

Cover

Figure 2c shows that, in Calderilla, Herradura and Lechagua, cover for *Ulva* sp. was larger on gametophytic than on tetrasporophytic thalli. However, these differences were only statistically significant for Calderilla and Lechagua where the lowest values were obtained (Fc=20.9836, $P<0.05$). The cover values for *Enteromorpha* sp. did not show differences between isomorphic phases nor locations (Fc=1.1108, $P>0.05$). The highest coverage for both epiphytes were registered in the control treatment.

Length

Ulva sp. thalli grew larger on the gametophytic thalli of *C. chamissoi* from Calderilla, Herradura and Lechagua (Figure 2e), although the only significant difference was seen for Calderilla population (Fc=12.9726, $P<0.05$). The maximum value was found on gametophytic thalli from P. Aldea (0.5±0.05 mm).

The differences observed in the lengths of *Enteromorpha* sp. thalli could not be explained individually by the different isomorphic phases or locations. These differences are related to an interaction between both factors (Fc=11.1462, $P<0.05$). The largest thalli of this epiphytes was also registered on the gametophytic thalli from P. Aldea (0.3±0.09 mm).

Discussion

Epiphytism continues to be one of the main problems in commercial production of marine algae in the world (Fletcher 1995; Oliveira et al. 2000; Ask and Azanza 2002; Lüning and Pang 2003) and *C. chamissoi* is not ane exception.

Our results show that zooids from both *Ulva* sp. and *Enteromorpha* sp. recruit on gametophytic and tetrasporophytic thalli of *C. chamissoi* in all the locations included in the study, although with different intensities.

In the case of *Ulva* sp., the number of thalli recruited on tetrasporophytic *C. chamissoi* was less

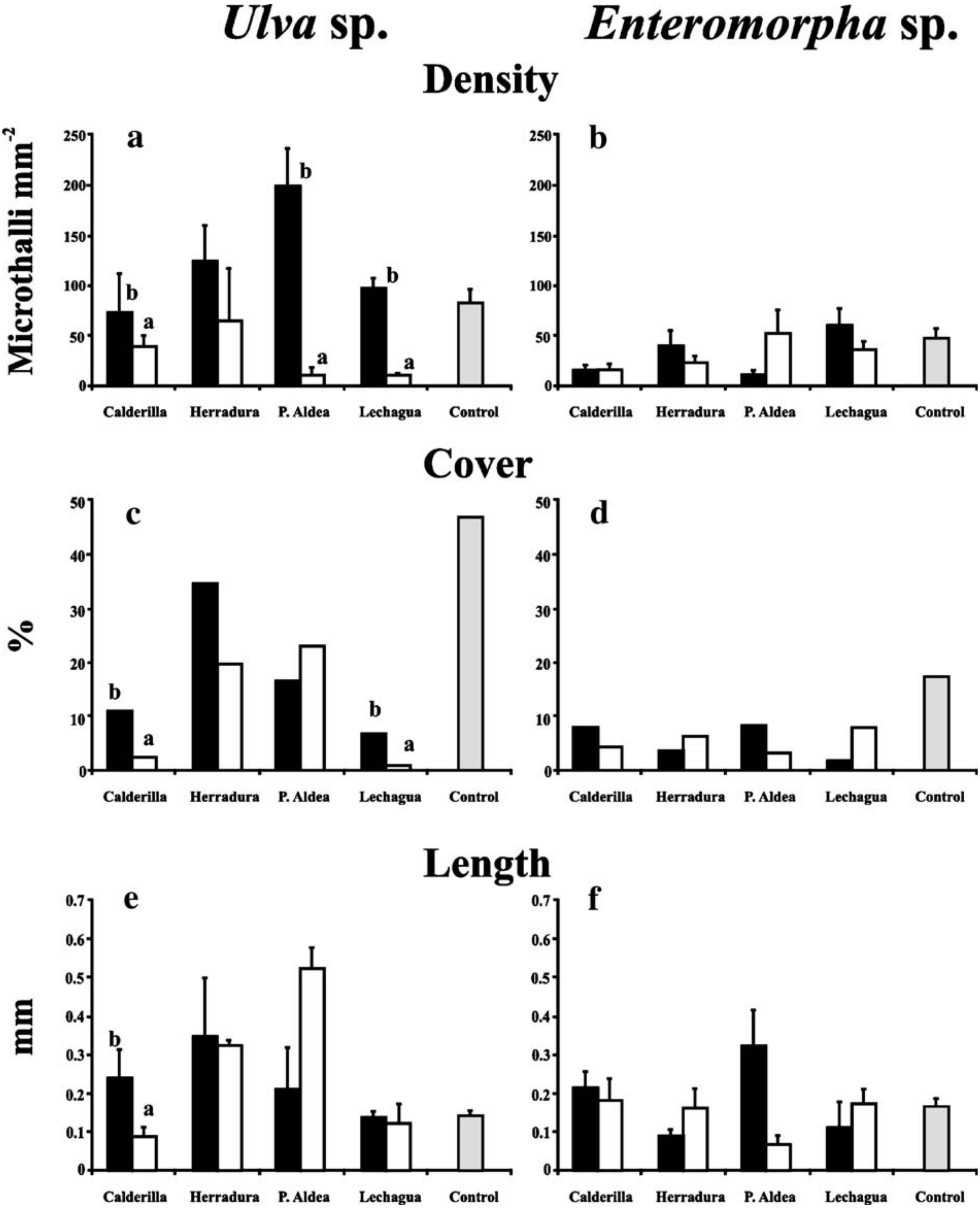

Figure 2 Density (a, b), cover (c, d) and length (e, f) of microthalli of *Ulva* sp. and *Enteromorpha* sp. recruits on gametophytic and sporopytic thalli of *Chondracanthus chamissoi* from four locations on the Chilean coast. *Black bars* female gametophytes, *white bars* sporophytes, *grey bars* control. Different *letters* above bars indicate significant differences between isomorphic phases.

than that registered for gametophytic thalli or for the control; this suggests that even for the tetrasporophytic thalli there was some kind of allelopathic interaction among the host and the epiphytes, reducing their recruitment.

It has been suggested that details in the morphology of the thalli could positively or negatively affect the attachment of epiphytes (Buschmann et al. 1992; Trono 1993; Bulboa 2001). Although Acleto (1986) reported a broad morphological variation in thallus morphology in populations of *C. chamissoi* from Peru, in this experiment the size and shape of the *C. chamissoi* thalli utilized were homogenized and morphological differences between gametophytes and sporophytes could not be detected.

Other possible explanations to explain the differences we obtained in the recruitment of epiphytes may be due to differences in the roughness of the thalli or the specific composition of the outermost cortical layer as remarked by Santelices (1992). We suggest that the differences in epiphyte resistance may better be attributed to the presence of κ-carrageenan in the gametophytes and λ-carrageenan in the sporophyte as has been shown to be the case for other members of the Gigartinaceae (Craigie and Leigh 1978).

The importance of the polysaccharide components of the cell walls in response to pathogen attacks was demonstrated for *Chondrus crispus* (Bouarab et al. 1999, 2001), where the type of carrageenan is important in the recognition of the attack, and in the defense response of the infected algae. Specifically, these authors found that the presence of κ-carrageenan in gametophytic thalli regulates the virulence levels of the green alga *Acrochaete operculata*, acting as an eliciting molecule of secondary metabolites triggered by the attack.

Many algal species produce chemicals that have inhibitory effects on certain fouling organisms (Hellio et al. 2002, 2004). Metabolites from some red algae inhibit settlement and germling development of gametes of the green alga *Ulva lactuca* (de Nys et al. 1995, Steinberg et al. 1998). Tissue growth, spore settlement, zygote formation and germlings of *Enteromorpha prolifera* were inhibited by methanol extracts of the brown algae *Ishige sinicola* and *Sargassum horneri* (Cho et al. 2001). Rothäusler et al. (2005) suggest the presence of a type of defensive response in *C. chamissoi* in the presence of attacking herbivores. However, defenses related to epiphyte susceptibility have not been described for this species. The results obtained with the tetrasporophytic thalli infected with *Ulva* sp. suggest that a mechanism, similar to that described by Bouarab et al. (1999, 2001) for *C. crispus*, may be involved in the response to epiphyte attachment.

Ulva sp. percent cover was also greater on gametophytic thalli when compared to tetrasporophytic thalli of *C. chamissoi* in at least three localities (Calderilla, Herradura and Lechagua). In all of the treatments, however, the percent coverage of the thalli with *Ulva* sp. was less than in the control treatment, with exceptionally low values on tetrasporophytic thalli from Calderilla and Lechagua.

A similar tendency was found in the size of the thalli of *Ulva* sp. that developed on tetrasporophytic thalli of *C. chamissoi* from Calderilla and Lechagua, which presented lower values than the other locations and the control treatment. The results obtained show that the tetrasporophytic phase from Calderilla and Lechagua present mechanisms that diminish recruitment of *Ulva* sp. as well as its subsequent development. This is reflected in the low values of cover and thallus size of the epiphytes.

Differences in physiological and ecological aspects as well as epiphytism susceptibility in *Gracilaria chilensis* have been attributed to the geographic origin of the algae (Santelices and Ugarte 1990; Buschmann et al. 1992). These differences are attributed to adaptations to the environment in the region of origin and may well be indicative of ecotypes, especially when there are clear clinal differences in each locality, as occurs along the Chilean coast. However, we did not investigate this aspect in this study.

Unlike what was observed in *Ulva* sp., the results obtained with *Enteromorpha* sp. did not show a clear pattern of epiphytism between the reproductive phases of the host.

Recently, Bulboa et al. (2005) showed that the presence of epiphytes on pilot cultivation areas of *C. chamissoi* could be an important problem in the commercialization of this alga for human consumption. All indications point to the search for epiphyte resistant strains as the best strategy by which to attack this problem (Ask and Azanza 2002; Lüning and Pang 2003). The results of this investigation demonstrate that tetrasporophytic thalli from Calderilla and Lechagua would be the best candidates for the cultivation of this species. However, it is necessary to find out if this resistance has a sustainable genetic base and if it is effective against other types of epiphytes that are common in northern Chile, such as some species of *Polysiphonia and Ceramium* that could attack our target species.

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References

Acleto C (1986) Algunos aspectos biológicos de *Gigartina chamissoi* (C. Ag.) J. Agardh (Rhodophyta, Gigartinales). Rev Cs Univ Nac San Marcos 74:33–47

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

Bouarab K, Potin P, Correa J, Kloareg B (1999) Sulfated oligosaccharides mediate the interaction between a marine red alga and its green algal pathogenic endophyte. Plant Cell 11:1635–1650

Bouarab K, Potin P, Weinberger F, Correa J, Kloareg B (2001) The *Chondrus crispus Acrochaete operculata* host-pathogen association, a novel model in glycobiology and applied phycopathology. J Appl Phycol 13:185–193

Bulboa CR (2001) Aspectos reprodutivos e biológicos de *Kappaphycus alvarezii* (Doty) Doty ex P. Silva e *K. striatum* (Schmitz) Doty (Gigartinales, Rhodophyta). Bases para introdução e cultivo de espécies exóticas no litoral brasileiro, Dissertation, Universidade de São Paulo

Bulboa CR, Macchiavello JE (2001) The effects of light and temperature on different phases of the life history in the carrageenan producing alga *Chondracanthus chamissoi* (Rhodophyta, Gigartinales). Bot Mar 44:371–374

Bulboa CR, Macchiavello JE, Oliveira EC, Fonck E (2005) First attempt to cultivate the carrageenan-producing seaweed *Chondracanthus chamissoi* (C. Agardh) Kutzing (Rhodophyta; Gigartinales) in Northern Chile. Aquac Res 36:1069–1074

Buschmann AH, Gómez P (1993) Interaction mechanisms between *Gracilaria chilensis* (Rhodophyta) and epiphytes. Hydrobiologia 261:345–351

Buschmann AH, Kuschel FA, Vergara PA, Schulz J (1992) Intertidal *Gracilaria* farming in southern Chile-differences of the algal proveniance. Aquat Bot 42:327–337

Cho JY, Kwon EH, Choi JS, Hong SY, Shin HW, Hong YK (2001) Antifouling activity of seaweed extracts on the green alga *Enteromorpha prolifera* and the mussel *Mytilus edulis*. J Appl Phycol 13:117–125

Craigie JS, Leigh C (1978) Carrageenans and agar. In: Hellebust JA, Craigie JS (eds) Physiological and biochemical methods. Cambridge University Press, Cambridge, pp 109–131

D’Antonio C (1985) Epiphytes on the rocky intertidal red alga *Rhodomela larix* (Turner) C Agardh: negative effects on the host and food for herbivores? J Exp Mar Biol Ecol 86:197–218

de Nys R, Steinberg PD, Willemsen P, Dworjanyn SA, Gabelish CL, King RJ (1995) Broad spectrum effects of secondary metabolites from the red alga *Delisea pulchra* in antifouling assays. Biofouling 8:259–271

Edwards P (1970) Illustrated guide to the seaweeds and sea grasses in the vicinity of Porto Aransas, Texas. Contr Mar Sci Suppl 15:1–228

Fletcher RL (1995) Epiphytism and fouling in *Gracilaria* cultivation: an overview. J Appl Phycol 7:325–333

Garcia-Jimenez P, Marian FD, Rodrigo M, Robaina MM (1999) Sporulation and sterilization method for axenic culture of *Gelidium canariensis*. J Biotechnol 70:227–229

González J, Meneses I (1996) Differences in the early stages of development of gametophytes and tetrasporophytes of *Chondracanthus chamissoi* (CAg) Kutzing from Puerto Aldea, Northern Chile. Aquaculture 143:91–107

González J, Meneses I, Vásquez JA (1997) Field studies in *Chondracanthus chamissoi* (C. Agardh) Kutzing: seasonal and spatial variations in life cycle phases. Biol Pesq 26:3–12

Harder T, Dobretsov S, Qian P (2004) Waterborne polar macromolecules act as algal antifoulants in the seaweed *Ulva reticulata*. Mar Ecol Prog Ser 274:133–141

Hayden HS, Blomster J, Maggs CA, Silva PC, Stanhope MJ, Waaland JR (2003) Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. Eur J Phycol 38:277–294

Hellio C, Berge JP, Beaupoil C, Le Gal Y, Bourgougnon N (2002) Screening of marine algal extracts for anti-settlement activities against microalgae and macroalgae. Biofouling 18:205–215

Hellio C, Marechal JP, Veron B, Bremer G, Clare AS, Le Gal Y (2004) Seasonal variation of antifouling activities of marine algae from the Brittany coast (France). Mar Biotechnol 6:67–82

Hoffmann A, Santelices B (1997) Flora Marina de Chile Central. Ediciones Universidad Católica de Chile, Santiago, Chile

Jennings JG, Steinberg PD (1997) Phlorotannins versus other factors affecting epiphyte abundance on the kelp *Ecklonia radiata*. Oecologia 109:461–473

Kuschel FA, Buschmann AH (1991) Abundance, effects and management of epiphytism in intertidal cultures of *Gracilaria* (Rhodophyta) in Southern Chile. Aquaculture 92:7–19

Lüning K, Pang SJ (2003) Mass cultivation of seaweeds: current aspects and approaches. J Appl Phycol 15:115–119

Oliveira EC, Paula J, Plastino M, Petti R (1995) Metodologias para el cultivo no axénico de macroalgas marinas *in vitro*. In: Alveal K, Ferrario ME, Oliveira E, Sar E (eds) Manual de métodos ficológicos. Universidad de Concepción, Concepción, pp 429–447

Oliveira EC, Alveal K, Anderson RJ (2000) Mariculture of the agar-producing Gracilarioid red algae. Rev Fish Sci 8:345–377

Ramírez ME, Santelices B (1991) Catálogo de las algas marinas bentónicas de la costa temperada del del Pacifico de Sudamerica. Monografías Biológicas 5

Rothäusler E, Macaya EC, Molis M, Wahl M, Thiel M (2005) Laboratory experiments examining inducible defense show variable responses of temperate brown and red macroalgae. Rev Chil Hist Nat 78:603–614

Santelices B (1992) Strain selection of clonal seaweeds. In: Round FE, Chapman DJ (eds) Progress in phycological research. Biopress, Bristol, pp 85–116

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

Santelices B, Varela D (1993) Exudates from *Gracilaria chilensis* stimulate settlement of epiphytic ulvoids. Hydrobiologia 261:327–333

Steinberg PD, de Nys R, Kjelleberg S (1998) Chemical inhibition of epibiota by Australian seaweeds. Biofouling 12:227–244

Trono G (1993) *Eucheuma* and *Kappaphycus*: taxonomy and cultivation. In: Ohno M, Critchley AT (eds) Seaweed cultivation and marine ranching. Japan International Cooperation Agency, Kanagawa, Japan, pp 75–88

Vásquez JA, Vega JM (2001) *Chondracanthus chamissoi* (Rhodophyta, Gigartinales) in Northern Chile: ecological aspects for management of wild populations. J Appl Phycol 13:267–277

Wheeler W, Neushul M, Harger B (1981) Development of a coastal marine farm and its associated problems. In: Levring T (ed) Proceedings of the Tenth International Seaweed Symposium. Walter de Gruyter, Berlin, pp 631–636