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Biomass, Reproductive Phenology and Chemical Characterization of Soluble Polysaccharides from *Rhodymenia howeana* Dawson, (Rhodymeniaceae, Rhodymeniales) in Northern Chile

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Monthly and bathymetric variation of biomass, reproductive phases and chemical characterization of soluble polysaccharides in a shallow subtidal *Rhodymenia howeana* population at La Herradura of Guayacán Bay, Coquimbo (Chile), has been monitored between July 1994 and August 1995. Overall productivity of the population did not change during the study period, although the wet weight increased significantly with depth. The species showed winter peaks in reproductive activity, whereas in summer the frequency of reproductive plants was near zero. Throughout the study period the tetrasporic phase predominated over gametophytic phases.

The non-fibrillar polysaccharides of *Rhodymenia howeana* were evaluated seasonally. Samples were sequentially extracted with water, acid and alkaline solutions and the extracts analysed by total hydrolysis, gas-liquid chromatography and infrared spectroscopy. The total yield of the extracts was 5.3% in winter, while in spring and summer values were close to 30%. The highest yields were obtained in aqueous and alkaline media and correspond mainly to a polysaccharide mixture of a sulphated galactan type and neutral glucans.

Introduction

Recent studies on the Rhodymeniales have focused on phylogenetic, taxonomic and life cycle issues (Maiz *et al.* 1987, Saunders and Kraft 1994, Strachan *et al.* 1995, Cáceres *et al.* 1997). Autoecological and reproductive aspects of most members of this Order in the natural environment have been ignored. With the exception of the pioneer works of Semesis and Dawes (1986), the chemical characterization of populations and the assessment of the quality of phycolloids in species of the Rhodymeniales have been largely neglected although many are abundant in shallow-sublittoral communities worldwide (Lee and Kurogi 1971, Lu *et al.* 1986, Kajimura 1987, Leonar and Clark 1993).

In Chile, 12 species of *Rhodymenia* have been recorded, this genus being distributed throughout the length of the continental coast and offshore islands (Ramirez and Santelices 1991). *Rhodymenia howeana* Dawson (Dawson 1941) is endemic to the South American west coast where it ranges from the Islas Chinchas, Perú (ca. 4° S) to Chiloé, Chile (ca 43° S). At the southern limit of its distribution, it is frequent in the rocky intertidal at exposed habitats (Santelices 1989). In northern Chile this species is common in shallow protected subtidal habitats growing on mixed substrata, and at La Herradura Bay, it grows on hard bottoms overlain with gravel and broken shells. Together with *Ulva* spp and *Chondrachantus chamissoi* (C. Agardh) Kützing, it accounts for over 90% of the total biomass at this locality (Salinas 1997).

Despite its frequency in shallow-water habitats, no data are available on the biology, ecology and chemical composition of this macroalgae. The present study attempts to determine the variation of biomass and reproductive phases as a function of time and depth and to characterize chemically the soluble polysaccharides of *Rhodymenia howeana* at La Herradura Bay in northern Chile.

Materials and Methods

Study area

La Herradura Bay is a horseshoe-shaped bay protected from southwest winds which are characteristic of the southern and central Chilean coast. The Bay of La Herradura is 3.27 km^2 in area, has a mouth 0.4 km wide, and its diameter at the widest portion is 0.93 km. *Rhodymenia howeana* coexists at the study area with *Ulva* spp and *Chondrachantus chamissoi* on hard bottoms overlain with gravel and broken shells. A bed of *Gracilaria chilensis* Bird, McLachlan *et* Oliveira, described in Santelices *et al.* (1984), is at the lower limit of the algal community.

Evaluation of biomass and reproductive phenology

Sampling was carried out between July 1994 and August 1995 along the south bank of La Herradura Bay (29°58'30" S, 71°22'30" W) (Fig. 1). Three permanent parallel transects, each 60 m long, and separated by a distance of 10 m were laid perpendicular to the shore and across the whole width of the algal community. These transects were sampled monthly by SCUBA diving. Sampling stations were positioned at 10 m intervals long the transects. Sampling stations at equal distances along each transect were treated as replicates because of the uniformity of the algal cover. To avoid the effects of previous quadrat sampling, every month the samples were taken one meter away from the previous one.

The sampling unit was a quadrat of 0.25 m^2. All the algae contained in it were collected, put into labelled plastic bags and, transported to the laboratory. The fronds of *Rhodymenia howeana* were separated from those of other algae, cleaned of invertebrates and epiphytes, weighed (90% humidity) and, separated by reproductive phase. Female gametophytes were identified by the presence of cystocarps, and tetrasporophytes were identified by the presence of tetrasporangial sori. Fronds without evident reproductive structures were considered as non-reproductive or male gametophytes, two categories that were lumped together. The biomass of each reproductive phase was expressed as a percentage of the total wet-weight per harvest per month. Percentage presences of the reproductive phases as a function of depth was determined between May and August 1995, the period of highest abundance of identifiable reproductive biomass. Biomass values were expressed in wet-weight g/m^2.

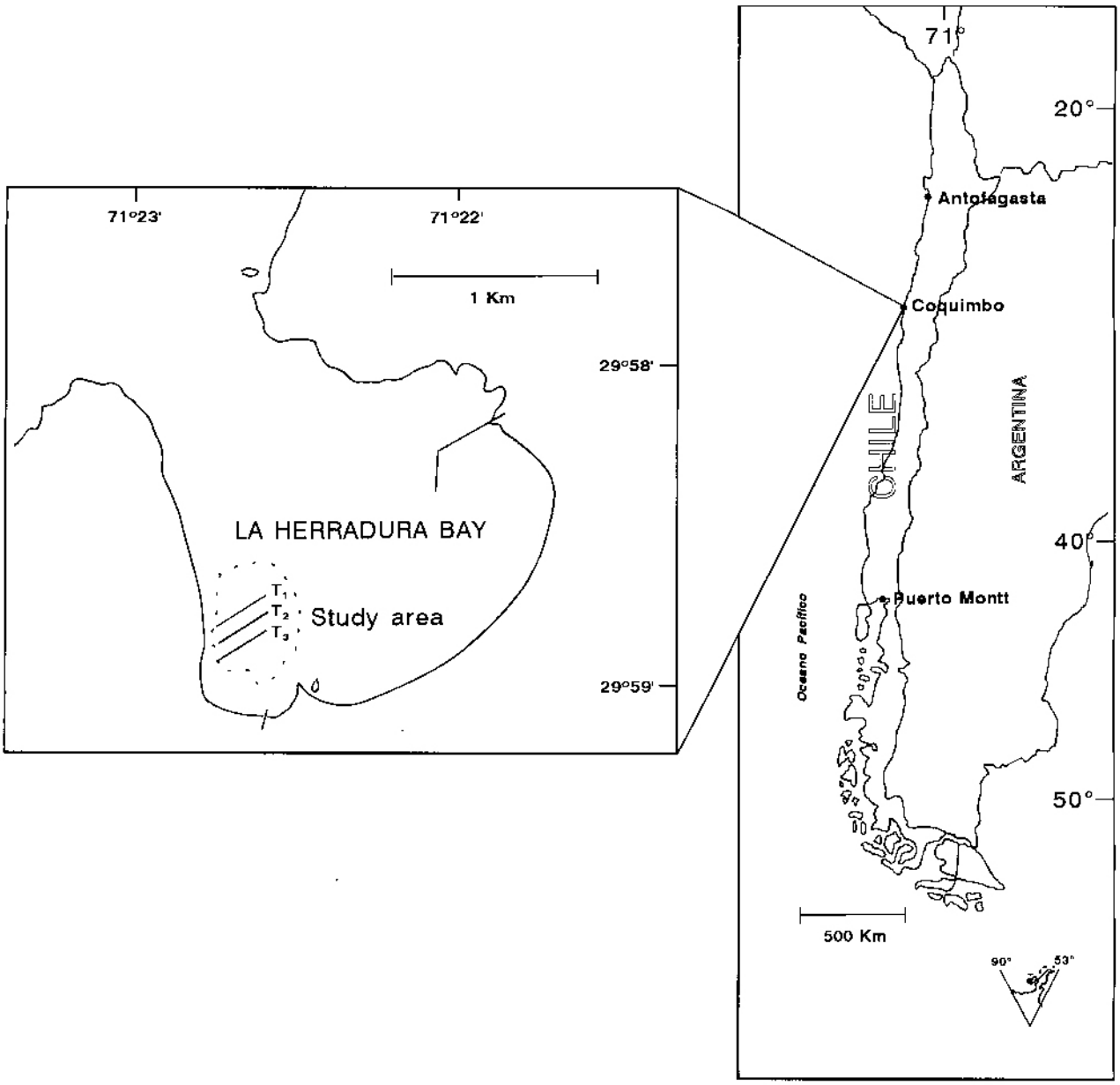

Fig. 1. Map of study area.

Methods of chemical characterization

Extractions of the phycocolloids were conducted on unsorted samples, no differentiation between reproductive phases, as the second derivate of the Fourier transform infrated spectroscopy (FT-IR) of dry tetrasporic and cystocarpic plants showed no differences in their characteristic absorptions bands (Caceres *et al.* 1997).

Solvent systems used for paper chromatography (PC) were (A) ethyl acetate-ethanol-water (8 : 2 : 1) and (B) pyridine-ethyl acetate-water (4 : 10 : 3). Gas-liquid chromatography (GLC) analysis was carried out in a Shimadzu GC-14B gas chromatograph equipped with a flame ionization detector using a fused silica gel capillary column (15 m × 0.25 mm) coated with Sp-2330, and performed with an initial 5 min hold at 150 °C and, to 210° for 10 min. The helium flow was 20 mL min^{-1}. Sulfate and nitrogen content were determined by microanalysis at the Facultad de Química y Farmacia, Universidad de Chile.

The FT-IR spectra of ground seaweeds and polysaccharide samples in KBr pellets (10% w/w) were recorded in the 4000–400 cm^{-1} region using a Bruker IFS 66*v* instrument.

Dried ground seaweed samples (400 g) were extracted with distilled water at 95 °C for 2.5 h then filtered through muslin. The filtrate was centrifuged and the supernatant dialysed against distilled water for two days, concentrated *in vacuo*, and poured into ethanol. The precipitate was separated by centrifugation, dissolved in a minimum volume of distilled water, and freeze-dried. The remaining seaweed extract was stirred for 3 days with 1 L of 0.2 M HCl at room temperature and centrifuged. The supernatant was dialysed against distilled water for 24 h, concentrated, and poured into ethanol. The precipitate after separation was dissolved in a minimum volume of distilled water and freeze-dried. The residue after acid extraction was stirred for 3 days with 1 L of 1 M NaOH at room temperature, and the extraction process was repeated. Finally, the residue was neutralized by stirring for 24 h with HCl, filtered, and extracted with distilled water at 95 °C for 3 h.

Each extract (0.020 g) was treated with 4 mL of 2 M trifluoroacetic acid at 90 °C for 16 h. Excess acid was removed by repeated evaporation with water. Aliquots of each hydrolysate were reduced and acetylated according to Wolfrom and Thompson (1963). The alditol acetates were analysed by GLC. The per-O-acetyl-alditols of D-xylose, D-galactose, D-glucose, D-mannose and L-rhamnose were used as standards.

The extract (0.5 g) was dissolved in 40 mL of distilled water and treated with an excess of 1% cetrimide (SIGMA) aqueous solution as described by Matsuhiro *et al.* (1996). The mixture was stirred overnight and centrifuged. The pellet was washed with distilled water, dissolved in 4 m NaCl aqueous solution, and precipitated into 5 volumes of ethanol. The precipitate was dissolved in water, dialysed against distilled water, concentrated *in vacuo* and freeze-dried.

One way ANOVA was used to determine differences between the distribution patterns and the Tukey Test was employed for *a prosteriori* multiple comparisons (Sokal and Rolf 1981). The Kruskall Wallis non parametric test for multiple comparisons was used when no hemoscedasticity of variances was detected (Sokal and Rolf 1981).

Results

During the study period, the monthly biomass of *Rhodymenia howeana* at La Herradura Bay did not show significant variation (Kruskall Wallis, $p < 0.05$). Nonetheless, despite the absence of seasonality, a considerable decrease of the total biomass was observed in January (Fig. 2).

The biomass of *Rhodymenia howeana* increased significantly with depth (Kruskall-Wallis, $p < 0.05$). At shallow depths the mean monthly biomass, throughout the sampling period, did not exceed 50 g/m^2. In contrast, the highest biomass occurred in the deepest sampling sites, where it reached values of over 300 g/m^2 at 6 m depth (Fig. 3).

The relative abundance of *Rhodymenia howeana* reproductive biomass showed a clear seasonal pattern

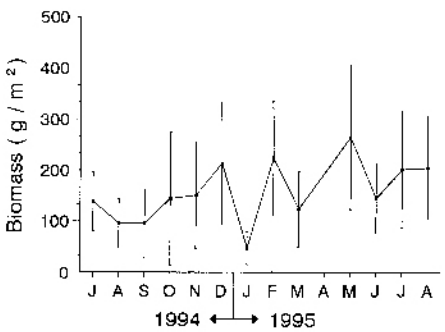

Fig. 2. Temporal variation of wet-weight biomass of *Rhodymenia howeana* at La Herradura Bay. (X ± 2SE)

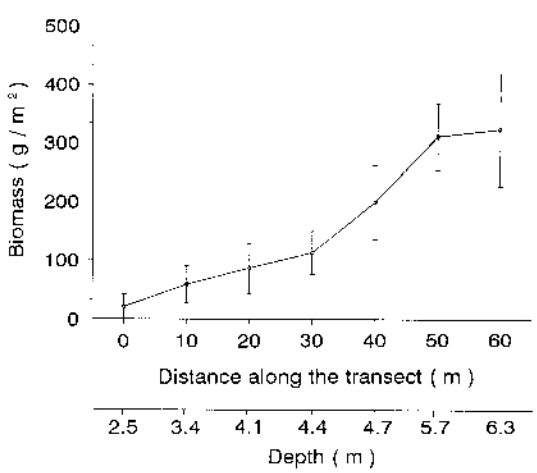

Fig. 3. Distribution of wet-weight biomass of *Rhodymenia howeana* with depth. (X ± 2SE).

over the study period (Fig. 4A). Plants with evident reproductive structures decreased towards the spring months and definitely disappeared during the summer months. In contrast, the greatest percentage of reproductive plants was seen during the autumn-winter period (Fig. 4A). During August and September, 1994, the rate of reproductive to vegetative biomass was approximately 1 : 1. From October in the same year, the vegetative biomass increased gradually to 100% of the biomass in January and February samplings (1995). Reproductive tissues could be first recognized in March, and from May to August, 1995, reproductive plants constituted over 70% of the total biomass collected (Fig. 4A). Tetrasporophytes were always more abundant than cystocarpic plants, both phases had abundance peaks in autumn and winter (Fig. 4B). If the months of greatest abundance of reproductive plants are considered (May–August 1995), tetrasporophytes are still significantly more abundant than cystocarpic and vegetative plants (Kruskall-Wallis, $p < 0.05$) (Fig. 5). In that period, the biomass of *Rhodymenia howeana* cytoscarpic and tetrasporic phases increased significantly with depth (Kruskall-Wallis, $p < 0.05$) (Fig. 6). In the depth range of 0 to 3–4 m no significant differences were detected in the relative biomass of both reproductive phases, whereas in the 4 to 6 m range differences between both phases were significant (Tukey Test, $p < 0.05$), with tetrasporophytes predominating (Fig. 6).

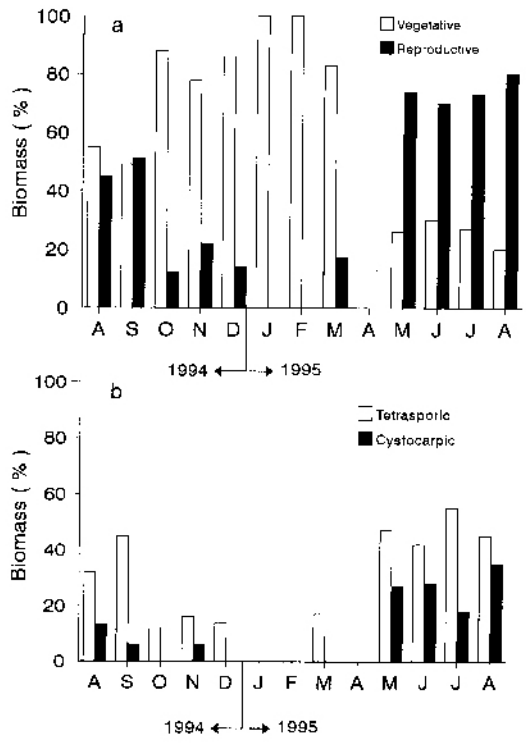

Fig. 4. Temporal variation of reproductive phases of *Rhodymenia howeana*: (a) Vegetative and total reproductive biomass; (b) Tetrasporic and cystocarpic biomass.

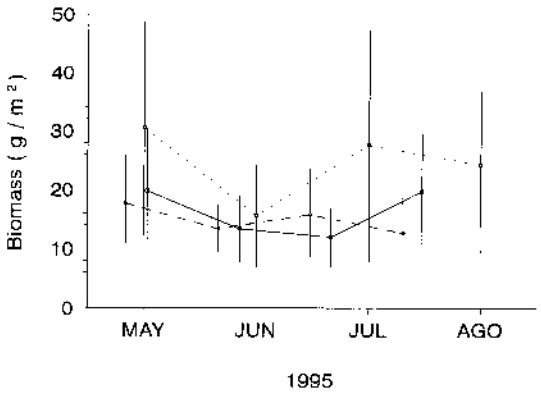

Fig. 5. Distribution of reproductive biomass of *Rhodymenia howeana* during months of higher abundance of plants. (– – vegetative; ···· tetrasporic; —— cystocarpic). (X ± 2SE)

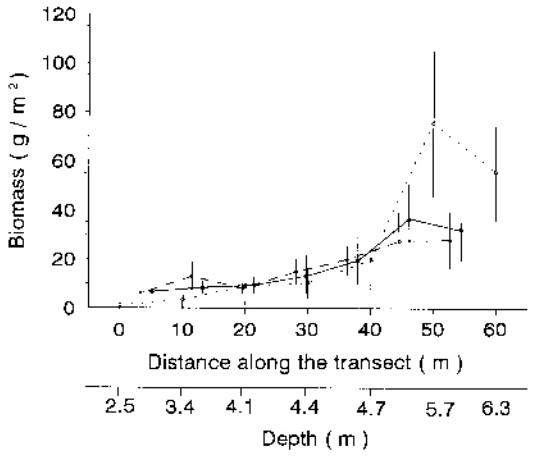

Fig. 6. Depth distribution of reproductive biomass of *Rhodymenia howeana*. (– – vegetative; ···· tetrasporic; —— cystocarpic). (X ± 2SE)

Yield and chemical composition of the polysaccharides extracted seasonally from unsorted *Rhodymenia howeana* are presented in Table I. Composition of the extracts was studied by total hydrolysis and GLC analysis of the alditol acetates. Molar ratios of the constituent monosaccharides were obtained from the GLC data. The alkaline extracts (ALE) from plants collected in spring and summer, as show by paper chromatography in systems A and B contained the monosaccharides galactose and glucose as major monosaccharides and xylose and mannose as minor constituents. Boths extracts were fractionated with cetrimide (Table II). The GLC analyses of the alditol acetates derived from the hydrolisates indicate that the soluble fraction of the alkaline extracts from spring collections is mainly a glucan. The content of nitrogen indicates that the soluble fraction of the spring alkaline extracts contains around 40% of protein. It is possible that the sulphur present in this fraction arises from the protein. Fractionation of the alkaline extracts in summer collections was not so effective. The insoluble fractions were mainly sul-

phated galactans bearing the sulphate group in the axial secondary position of carbon 4 according to the FT-IR spectra (absorption at 844 cm^{-1}). The soluble fraction is composed of a glucan contaminated with a sulphated polysaccharide.

Discussion

Rhodymenia howeana at La Herradura Bay is primarily distributed at 2 to 6 m depths with the largest biomasses found between 4 to 6 m. Its lower distribution limit is probably conditioned by the availability of adequate hard substrata. The scarcity of the plants at shallower depths may be due to their response to factors like irradiance and temperature. Other algae like *Ulva* spp. and *Chondracanthus chamissoi* coexist with *Rhodymenia howeana*, particularly at shallow depths. It is likely that these opportunistic or more competitive species would displace *Rhodymenia howeana* from shallower areas, thus reducing the primary sub-

Table I. Yields and constituents of the extracts.

Extract	Yield %	SO_3Na %	N %	Molar ratio Xyl : Man : Gal : Glu
Winter				
AQE	1.7	24.01	2.844	0.00 : 0.00 : 0.39 : 1.00
ACE	1.3	25.68	2.132	0.00 : 0.00 : 1.69 : 1.00
ALE	1.9	15.20	7.452	0.00 : 0.00 : 8.77 : 1.00
ANE	0.5	13.08	6.296	0.00 : 0.00 : 6.37 : 1.00
Spring				
AQE	10.2	16.28	4.255	0.00 : 0.00 : 10.56 : 1.00
ACE	6.7	24.89	1.420	0.00 : 0.00 : 8.07 : 1.00
ALE	10.1	19.79	4.807	0.00 : 0.00 : 2.03 : 1.00
ANE	1.5	27.51	1.986	0.00 : 0.00 : 9.65 : 1.00
WNE	2.8	17.20	3.991	
Summer				
AQE	8.6	13.45	4.881	0.67 : 0.00 : 9.33 : 1.00
ACE	5.1	23.63	2.913	0.00 : 0.00 : 7.68 : 1.00
ALE	9.6	13.59	4.994	0.00 : 0.00 : 11.99 : 1.00
ANE	0.6	15.48	2.736	0.00 : 0.00 : 0.44 : 1.00
WNE	6.0	5.39	2.860	0.00 : 0.00 : 0.00 : 1.00
Autumn				
AQE	9.6	18.39	3.989	
ACE	4.8	26.65	0.875	0.00 : 0.00 : 3.36 : 1.00
ALE	3.5	13.97	6.804	0.00 : 0.45 : 2.15 : 1.00
ANE	0.5	10.19	6.252	0.00 : 0.00 : 1.36 : 1.00
WNE	0.6	16.94	5.606	0.00 : 0.00 : 1.00 : 0.00

N : nitrogen, Xyl: xylose, Gal: galactose, Glu: glucose, Man: mannose.
AQE: Aqueous extract, ACE: acid extract, ALE: alkaline extract, ANE: neutralised aqueous extract, WNE: neutralizing water.

Table II. Yields and constituents of the fractions obtained by treatment with cetrimide.

Extract	Yield %	SO_3Na %	N %	Molar ratio Xyl : Man : Gal : Glu
Spring ALE				
Soluble	13.20	1.450	6.420	0.00 : 0.00 : 00.0 : 1.00
Insoluble	46.30	10.060	4.023	0.10 : 0.07 : 1.00 : 0.20
Summer ALE				
Soluble	21.80	5.498	8.600	0.21 : 0.29 : 1.00 : 0.82
Insoluble	50.36	9.310	4.622	0.10 : 0.06 : 1.00 : 0.00

N : nitrogen, Xyl : xylose, Gal: galactose, Glu: glucose, Man: mannose, ALE: alkaline extract.

stratum for spore settlement. A third factor is the association between *Rhodymenia howeana* and the urochordate *Pyura chilensis* Molina (personal observation J. Vásquez). The rugose tunic surface of *Pyura chilensis*, would help spore settlement, and the nutrients from its excretion processes, could contribute to maximize the biomass in areas where *Pyura* abounds. The distribution of *Pyura chilensis* from 4 to 6 m depth exactly corresponds to the maximum abundance of *Rhodymenia howeana* in the study area. This cause and effect relationship has yet to be well established.

Rhodymenia howeana did not show significant variation in biomass during the study period. This contrasts with the marked seasonality of abundance shown by other red algae from temperate subtidal environments of the South American Pacific coast and other parts of the world (Norall *et al.* 1981, Santelices 1989, Mathieson 1989, Piriz 1996). The stability of the surface temperature values in the study area (13.1 ± 0.02, Salinas 1997) could be a decisive factor governing the absence of temporal patterns in the total biomass measurements of *Rhodymenia howeana*. The decrease of biomass that took place during January 1995, may have been a consequence of water movement during the summer period that produces a high mortality among larger plants.

In contrast to the absence of temporal patterns in total biomass, the reproductive phenology of *Rhodymenia howeana* showed marked seasonal variation. The maximum biomass of reproductive plants occurred in winter, whereas reproductive plants were totally absent during summer. Seasonal variations in the reproductive events of macroalgae usually respond to changes in environmental factors such as photoperiod, irradiance and temperature, the interaction of which determines the initiation and development of reproductives structures (Lüning 1990). In winter the surface temperatures at La Herradura Bay fluctuate between 12 °C and 14 °C, irradiance ranges between 75 and 80 Watt m^{-2}, and the photoperiod of this latitude corresponds to 11 : $\overline{13}$ h light−darkness (Moraga and Olivares 1987, Anonymous 1995).

Over the study period, tetrasporophytic plants predominated in the population of *Rhodymenia howeana*. An imbalance of one phase over the other seems to be a common occurrence in isomorphic red algae, and a number of hypotheses have been suggested to explain the disproportion between phases (Whittick 1978, Kilar and Mathieson 1978, Mathieson 1989). The vertical distribution of the haploid and diploid reproductive phases was bimodal with both phases occurring at between 2 and 6 m depth but tetrasporophytes predominated at between 4 and 6 m depth. This phenomenum, in which life-history phases differ in abundance at different depths has been described for *Callophyllis cristata* (Linnaeus) Kützing, *Membranoptera alata* (Hudson) Stackhouse and *Phycodris rubens* (Linnaeus) Batters, and predominance of the tetrasporophyte with increasing depth has been described for *Chondrus crispus* Stackhouse and *Ptilota serrata* Kützing (Norall *et al.* 1981, Mathieson 1989).

Regeneration of fronds and vegetative reproduction could explain the disproportion between phases detected in *Rhodymenia howeana* at Bahía La Herradura. Field observations suggest that the perennial stoloniferous basal system is capable of regenerating new fronds and that detached blade fragments can initiate stolons which can secondarely attach and produce a new plant. Both frond regeneration and vegetative propagation from drifting fragments are frequent in other red algae such as *Chondrus crispus*, *Gigartina acicularis* (Roth) Lamoroux and *Gigartina skottsbergii* Setchell *et* Gardner (Braga 1990, Mathieson 1989, Piriz 1996).

Extraction of the phycocolloids for chemical characterization was conducted from unsorted samples, since the second-derivative FT-IR spectra of dry tetrasporic and cystocarpic plants showed identical absorptions bands characteristics of carrageenophytes (Cáceres *et al.* 1996). The yield obtained in the aqueous extractions is much lower than those usually reported for the carrageenan and agar produced by red seaweeds (Ayal and Matsuhiro 1987, Matsuhiro and Urzúa 1990, 1991, 1992). Sequential extraction of the seaweeds residues remaining after removal of the water-soluble polysaccharides led to isolation of various sulphated galactans and glucans. The total extracts accounted for about 30% of the seaweed dry weight in spring 1994, summer and autumn 1995. The yield obtained in winter 1994 was very low, but over a period of 12 months the total yields (27.1%) became similar to those found for the other seasons.

The second-derivated FT-IR spectra of all the extracts showed no bands around 930 cm^{-1} indicative of 3,6-anhidrogalactose residues, neither was the diagnostic band of agar at 790 cm^{-1} present (Matsuhiro and Rivas 1993). In the region 845−836 cm^{-1} the characteristic band assigned to sulphate groups attached to the axial hydroxyl group on C-4 galactose residues was cleary present (Matsuhiro 1996). In this region, bands assigned to sulphate groups attached to primary and secundary equatorial hydroxyl groups were not found. From these results the presence of carrageenan type and agar type polysaccharides is excluded. No seasonal variation was found in the content of sulphate in the aqueous extracts, the values being similar to those reported for carrageenans (Ayal and Matsuhiro 1986). It is noteworthy that the content of nitrogen in the polysaccharides was highest in those obtained by extraction with alkaline solutions. Paper chromatography of the hydrolysates showed that all the extracts are composed mainly of galactose and glucose. The composition of the extracts differs from the carrageenan reported for *Rhodymenia pseudopalmata* (Lamouroux) Silva by Semesis and Dawes (1986). Sulphated galactans that do not show the characteristic features of agar and

carrageenans have been reported for the red seaweeds from different Orders. In 1978, Usov *et al.*, by sequential extraction of the red seaweed *Rhodymenia stenogona* Perestenko, now known as *Palmaria stenogona* Perestenko (Perestenko), from the Order Palmariales, isolated neutral xylans and a sulphated galactan devoid of 3,6-anhydro residues with a 10% of sulfate content. The major water soluble polysaccharides extracted from red seaweeds of the Order Palmariales are neutral xylans. The xylans are usually found together with sulphated galactans and/or mannans (Usov *et al.* 1981, Matulewicz *et al.* 1992). Recently, in the study of *Palmaria decipiens* (Reinsch.) Ricker, Matsuhiro and Urzúa (1996), found that the fraction of the polysaccharide that gave a complex with the detergent was an acidic cylogalactan linked covalently to a protein. Chemical analysis of the deproteinated polysaccharide showed that it is composed mainly of an α(1→3)-galactan partially subtituted by xylose, uronic acids and sulphated residues in positions 4 and 6. It is shown in Table II that the fraction of the summer alkaline which did not complex with cetrimide contained significant amounts of xylose and mannose. Probably, these monosaccharides arise from a separate polysaccharide.

On the basis of chemical and sepctroscopy analysis it can be concluded that *Rhodymenia howeana* products a rather complex mixture of sulphated galactans and a considerable amount of neutral glucans.

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