Phycologia Volume 56 (5), 579–589 Published 29 June 2017

Heteromorphic phases of *Leathesia marina* (Ectocarpales, Ochrophyta) over time from northern Patagonia, Argentina

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Abstract: The emergence and evolution of the heteromorphic life history in algae has been considered as a response to the selective pressures of changing environments. The brown alga *Leathesia marina* has a typical heteromorphic life history, alternating between microscopic branched filaments (gametophytic phase) and a macroscopic erect stage (sporophytic phase). The first aim of this study was to describe vegetative and reproductive morphologies of sporophytes (macrothalli and diploid phase) of *L. marina* and their relationship with environmental factors in an annual cycle. The second aim was to report the discovery of gametophytes (microthalli and haploid phase) in the natural environment on northern Patagonian coasts, and to describe their morphology, abundance and reproductive output. The macroscopic phase was observed over 8 months, from September to April during the warm season. The microscopic phase was observed in two periods, March–April and September–October. A temporal overlap was observed between the microscopic and macroscopic phases. The population of macrothalli had a type III survivorship curve. The cover, density and size of macrothalli were associated with warmer months, but the abundance of microthalli and reproductive output of both phases were higher in temperate periods. Demographic parameters, including mortality rate, survivorship, reproductive output and the morphological characteristics of *L. marina* are used to evaluate the adaptation of this species to a changing environment, typical of the temperate coasts of northern Patagonia in Argentina.

Key Words: Abundance, Macroalgae, Macrothalli and spacial microthalli, Morphology, Survivorship, Reproductive output

INTRODUCTION

In marine environments, especially rocky intertidal zones, algae are subject to pressures from the physical environment, competition with other algae and predation by herbivores (e.g. Littler & Littler 1980; Lubchenco & Cubit 1980; Garbary 2007). These pressures are reflected in a wide variety of adaptations on marine organisms that influence the evolution of life cycles, morphologies, phenological patterns, population dynamics and survival probabilities (Istock 1967).

The emergence and evolution of the heteromorphic life cycle comprise the sequential development of distinct stages in the same algal species with marked differences in morphology (Littler & Littler 1980). Seaweeds with heteromorphic life cycles exhibit a high degree of independence and differentiation between the different stages. Each phase has unique ecological and evolutionary constraints, and must be able to survive and reproduce (Schiel & Foster 2006). One advantage of the heteromorphic life cycle is that the two phases can exploit ecological niches that differ in temperature, day length, competitors or herbivore pressures (Lubchenco & Cubit 1980; Zupan & West 1990; Cunningham *et al.* 1993).

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DOI: 10.2216/16-117.1

The spacial–temporal differentiation between the two generations using the Lotka–Volterra competition model between species was studied by Hughes and Otto (1999) (Lotka 1925; Volterra 1926). These authors determined that niche differentiation between the two phases was necessary to prevent outcompetition of one phase by the other, thereby avoiding the loss of one of the two stages.

In many marine macroalgal species, one life stage is more tolerant to unfavourable environmental conditions (Couceiro *et al.* 2015). Red algae and brown algae often show ecological differences between a resting stage resistant to biotic or abiotic stress and a fast-growing ephemeral stage, which appears when seasonal conditions are suitable (Carney & Edwards 2006; Vergés *et al.* 2008).

Many seaweed species rely on microscopic stages to survive periods of environmental stress, and produce new macroscopic stages when conditions improve (Hoffmann & Santelices 1991; Edwards 2000). The microscopic stages tend to be more tolerant to unfavourable light conditions, temperature and nutrients (Chapman & Burrows 1971; Nakahara 1984; Wiencke & Dieck 1989, 1990). In brown algae with annual population dynamics, the prolonged development of microscopic stages is critical, because the macroscopic thalli may be absent for several months (McConnico & Foster 2005; Schiel & Foster 2006).

Inagaki (1958) classified *Leathesia* into two sections: section *Leathesia* and section *Primariae*. *Leathesia marina* (Lyngbye) Decaisne presents a medullary structure typical of

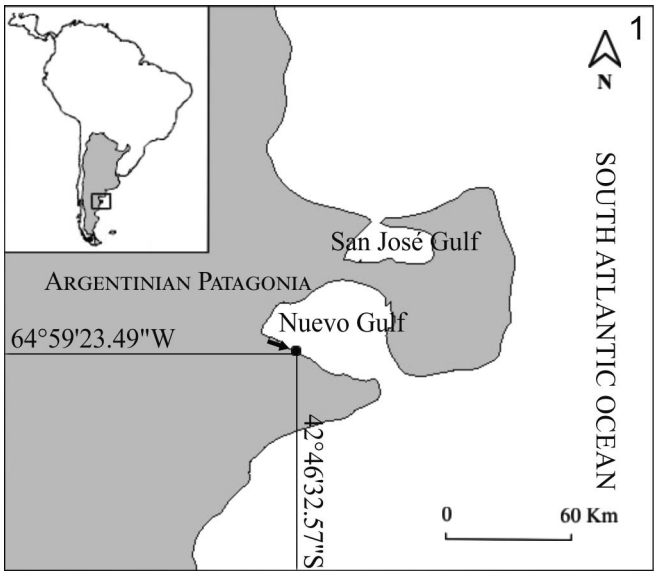


Fig. 1. Study area on the east coast of Nuevo Gulf, northern Patagonia, Argentina.

specimens of the section *Leathesia*. This differs from section *Primariae* on the basis of the presence of irregular medullary cells, and anastomoses in both the lower and middle regions, as well as a dichotomous arrangement at the top with rounded or elongated cells (Fritsch 1945; Fletcher 1987; Womersley 1987). In contrast, the section *Primariae*, as represented by *Leathesia primaria* Takamatsu, has long, elliptical, tightly adhering medullary cells that lack anastomoses (Takamatsu 1939; Inagaki 1958). However, the similarity in morphology between the two sections can hamper identification. Several studies revealed the presence of cryptic species with similar morphologies or cryptic states with heteromorphic life cycles that can result in false hypotheses of speciation (Peters *et al*. 2015). For this reason, a molecular study is being conducted to identify the current population and assess the possibility of finding cryptic species (iBOL Project, CONICET, Argentina; MAMA project: marine macroalgae from northern Patagonia of Argentina).

Leathesia marina is a cosmopolitan macroalga found in rocky intertidal communities from temperate to cold climates. This species has a typical heteromorphic life cycle, alternating between microscopic branched filaments (gametophytic phase) and a macroscopic erect stage (sporophytic phase) (Sauvageau 1925; Kylin 1933; Dangeard 1965; Peters 1987). Few studies have related the characteristics and dynamics of the life cycle of *L. marina* to phenological patterns and morphologies (Chapman & Goudey 1983; Quartino & Boraso de Zaixso 1996). Therefore, in this study, we evaluated the notion that phenological patterns could be regulated by the changing annual environment. Accordingly, our first aim was to describe in detail the vegetative and reproductive morphologies of *L. marina* sporophytes (macrothalli and diploid phase) and their relationship to a seasonal environment. The second aim was to report the discovery of gametophytes (microthalli and haploid phase) in the natural environment on northern Patagonian coasts and to describe their morphology, abundance and reproductive output.

MATERIAL AND METHODS

The sampling was carried out on the east coast of the Nuevo Gulf, located in northern Patagonian coasts (42°46′S, 62°59′W) (Fig. 1), from December 2013 to November 2014. The region is arid, and characterized by extreme weather conditions, with a predominance of strong west winds and year-round low humidity (Paruelo *et al*. 1998). It has a mean annual precipitation of 239 mm and a mean annual temperature of 13.4°C. Westerly winds have an annual mean speed of 16.6 km h^{-1} and reach 90 km h^{-1}. These strong dry winds, combined with low rainfall, give the Patagonian intertidal zone the highest desiccation stress recorded for rocky shore communities (Bertness *et al*. 2006). The seawater temperature ranged from 17.5°C in summer to 9.8°C in winter, with an annual average of 13.4°C. Day length varies between 15.2 h in summer and 9.1 h in winter. Solar radiation is maximum in summer (8342 W m^{-2}) and minimum in winter (1584 W m^{-2}). The tidal regimen is semidiurnal, with a maximum amplitude of 5.86 m and an annual average of 4.13 m (SHN 2014). In this study, environmental parameters, such as seawater temperature, day length and solar radiation, were provided by the Automatic Meteorological Station of the Climatology Laboratory of CENPAT-CONICET.

The substrate at the intertidal zone consists of a consolidated limestone platform, known locally as "tosca" (Casal 1946), and is characterized by numerous tidal pools. The shores are mostly covered at mid-level by the tiny mussels *Brachidontes rodriguezii* d'Orbigny (1842) and *Perumytilus purpuratus* Lamarck (1819); the lower level is dominated by *Corallina officinalis* Linnaeus. Fronds of *Leathesia marina* grew predominantly in the lower level of the intertidal zone.

To evaluate population dynamics, six quadrats (25 cm × 25 cm) were randomly placed in the low intertidal zone and photographed every 15 days throughout the year. We determined different demographic parameters, including density, cover, growth rate and life history, without removing the macrothalli. To estimate the cover of *Leathesia marina* when macrothalli were present, the photographs were analysed using ImageJ 1.46v image processing software (National Institutes of Health, Bethesda, Maryland, USA).

The survivorship data were used to construct a cohort life table (Deevey 1947), which was constructed by sampling individuals for 120 days at intervals of 15 days, from January to April. The remaining months were not considered since *Leathesia marina* was not present as macrothalli during that time. The coordinates of each individual in each photograph of each quadrat were recorded. Mortality was measured directly by counting the losses of individuals that had been present in a previous sampling period.

The microthallus stages grew associated with macrothalli of *Leathesia marina*. Microthallus abundance was calculated on the basis of the presence or absence of thalli on a 3 mm ×

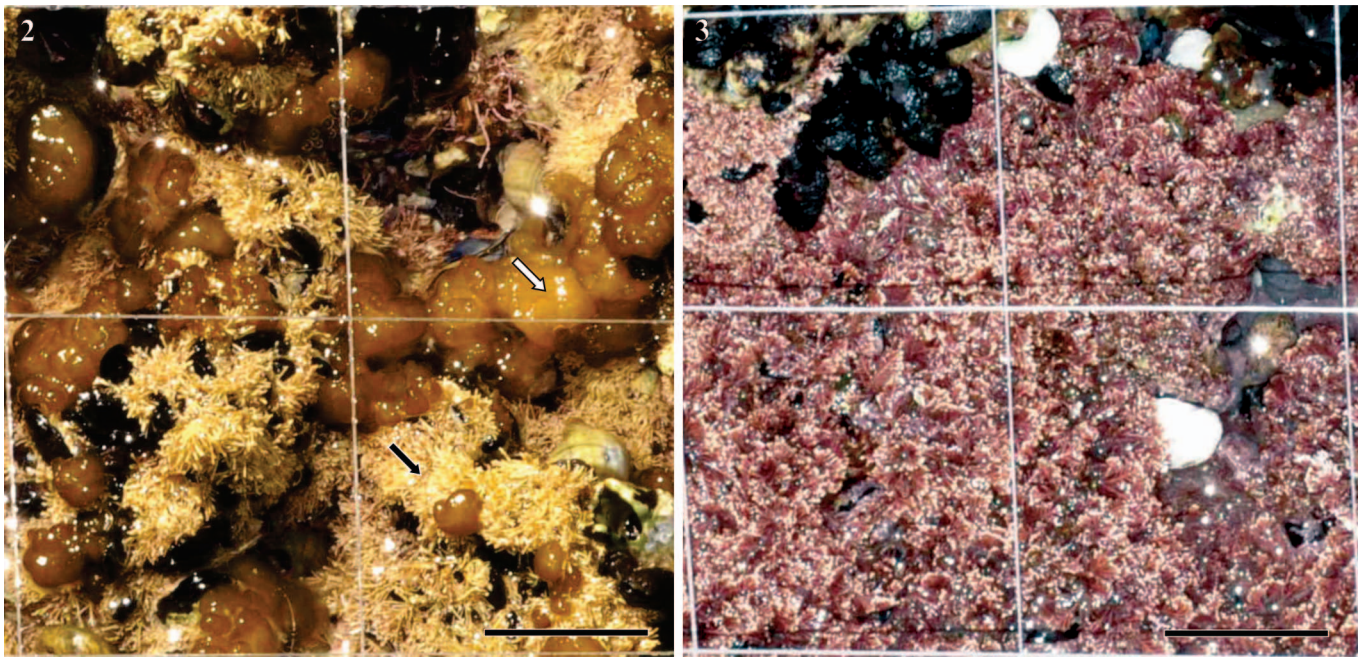


Fig. 2. Macrothalli of *Leathesia marina* (white arrow) epiphytic on *Corallina officinalis* (black arrow). Macrothalli young with globose shape and old macrothalli with rugose structure. Photograph taken in January 2014. Scale bar = 5 cm.
Fig. 3. Photograph of the same quadrat taken in June 2014 showing bed of *Corallina officinalis*. Scale bar = 5 cm.

3 mm section on 20 macrothalli collected at random each month.

To describe both vegetative and reproductive structures of the two phases of *Leathesia marina,* 20 macrothalli and associated microthalli were randomly collected each month. Samples were stored in plastic bags overnight at 5°C and later fixed in formaldehyde–glacial acetic acid–ethanol solution, at 8:1:1. Three vegetative morphological variables were measured on each macrothallus: maximum diameter, height and drained fresh mass. From these thalli, small sections of 3 mm × 3 mm were randomly selected for subsequent examination with a light microscope. Additional internal vegetative structures were measured: length and width of subcortical and medullar cells (first, second and third order), length and width of assimilatory filaments and width of hairs. The reproductive structures measured on each thallus included: number, length and width of unilocular and plurilocular sporangia; and number of loculi in biseriate and uniseriate plurilocular sporangia. On the microthalli, the variables measured were: length and width of the cells in prostrate filaments; number, length and width of plurilocular gametangia and number of loculi.

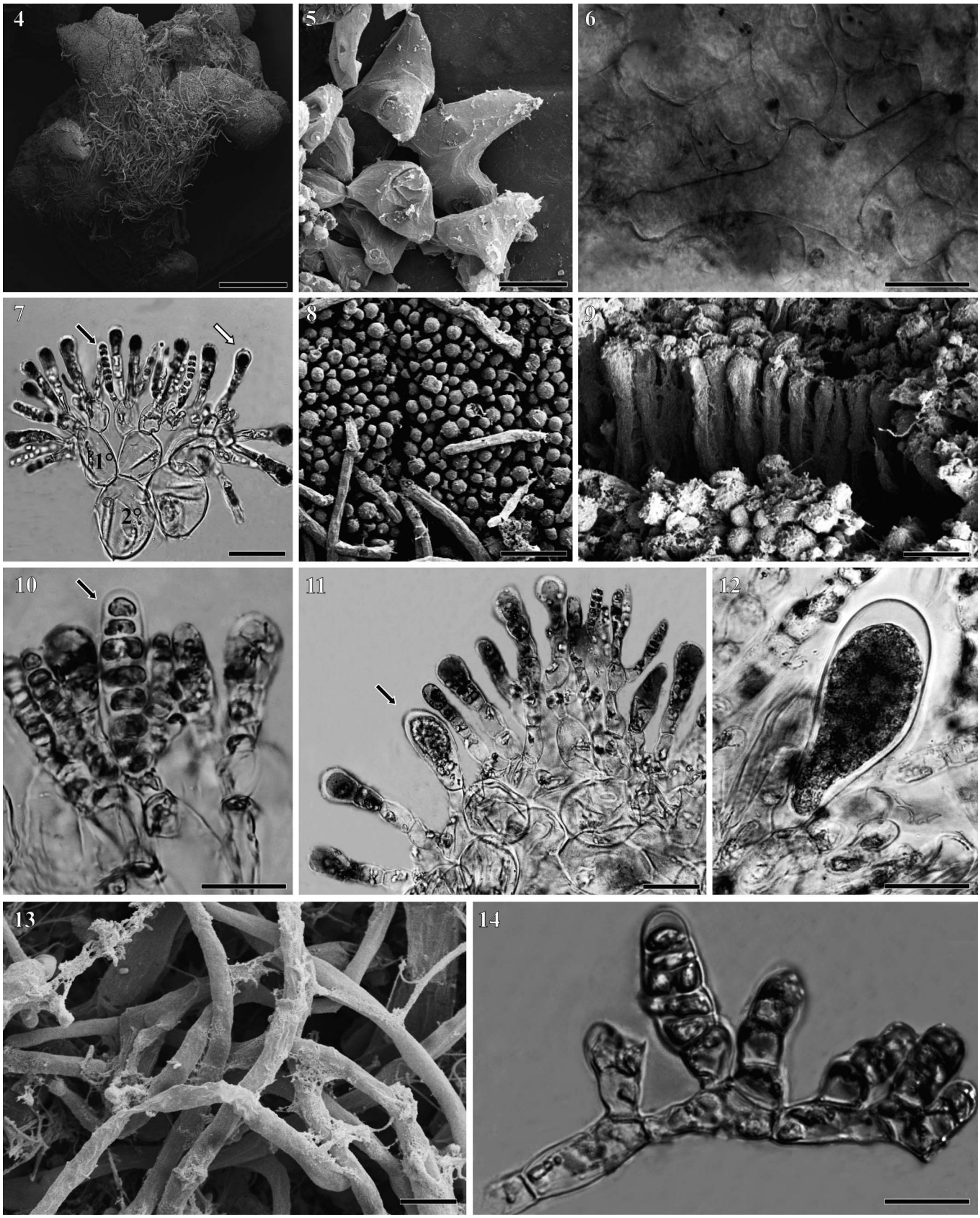
The reproductive output for both macrothalli and microthalli on 20 specimens was calculated. For this purpose, five transects of 125 µm *per* thallus (total of 625 µm *per* thallus) were randomly chosen each month, where the unilocular and plurilocular sporangia in macrothalli and gametangia in microthalli were counted using light microscopy at ×400 magnification.

To confirm the identity of the microthalli associated with the macrothalli of *Leathesia marina*, *in vitro* cultures were established. For this purpose, reproductive fragments of microthalli were sectioned and gently rinsed three times with sterile seawater. The fragments were placed in test tubes with a coverslip on the bottom, containing seawater enriched with modified Provasoli medium (Provasoli 1968) at 20°C with a light:dark regime of 16:8 h to simulate summer conditions, with a light irradiance of 25 µmol m^{-2} s^{-1} provided by cool white fluorescent tubes and monitored using a quantum flux meter (Apogee MQ-200, Logan, Utah, USA). The growth of the zygotes attached to the coverslip was monitored. Under these conditions, gamete fusions were observed. Over the course of 45 days the fused gametes from the gametophyte of *L. marina* formed mature macrothalli (sporophytic phases) with reproductive structures.

For scanning electron microscopy (SEM), fragments of macro- and microthalli were fixed in 2.5% glutaraldehyde containing 0.01 M sodium cacodylate buffer (pH 7.2) at 5°C for 2 h. Subsequently, three washes of sodium cacodylate buffer (0.005 M) were carried out for 10 min each. The fixed portions were dehydrated in an acetone series, following the protocol by Cáceres (1995). Finally, the samples were critical-point dried for 1 h, and were coated with gold in a sputter coater 9100 model 3 (Pelco, Clovis, California, USA) according to Sorrivas de Lozano & Morales (1986). Samples were observed with a Leo Evo 40 SEM (Jena, Germany).

Each data set was examined for homogeneity of variances using Barlett's test and normality using the Shapiro–Wilk test. To evaluate the differences in the population of *Leathesia marina*, the vegetative and reproductive parameters of the macrothalli and microthalli were examined using analyses of variance followed by multiple comparisons using Tukey's honestly significant difference.

The survivorship curves were adjusted using the Weibull (1951) distribution model:



Figs 4–14. Light and electrons microscope images of microthalli and macrothalli of *Leathesia marina*.
Fig. 4. SEM photograph of macrothalli showing irregular cushion-shaped morphology with numerous interstices.
Fig. 5. SEM photograph of macrothallus showing third-order medullar cells showing an irregular shape. Scale bar = 10 μm.

$$F(x;k;\lambda) = 1 - e^{-(x/\lambda)^k}. \quad (1)$$

The main advantages of this model are its simplicity and the ability to describe survivorship curves concave downward ($k > 1$) and convex upward ($k < 1$), when ($k < 1$) indicates an intense early mortality (Wilson 1994).

The relationships among the vegetative and reproductive variables of macrothalli of *Leathesia marina* and environmental parameters seawater temperature, day length and solar radiation were analyzed using principal component analysis (PCA) and Spearman correlation coefficients with standardized data. The statistical programs R Studio (R Core Team 2014) and PRIMER (Plymouth routines in multivariate ecological research) (Clarke & Warwick 2001) were used at significance level of 0.05.

RESULTS

Macrothalli of *Leathesia marina*

Macrothalli of *L. marina* were found from December 2013 to April 2014 and from September to November 2014, corresponding to moderate and warm seasons along the northern Patagonian coast. The population of *L. marina* was predominantly in the low intertidal zone. Only a few thalli were found at middle and upper levels. The low intertidal zone was colonized by invertebrates, including *Brachidontes rodriguezii* and *Perumytilus purpuratus,* and associated with a macroalgal community of *Corallina officinalis*, *Undaria pinnatifida* (Harvey) Suringar, *Myriogloea major* Asensi, *Ralfsia verrucosa* (Areschoug) Areschoug and occasionally *Adenocystis utricularis* (Bory) Skottsberg. *Leathesia marina* was often found as an epiphyte on beds of *C. officinalis* (Figs 2, 3).

Young macrothalli of *Leathesia marina* were globose and then expanded horizontally to become a rugose thallus (Figs 2, 4). Maximum diameter, height and drained fresh mass were significantly different among months (Table 1). Thallus diameter ranged from 2.9 ± 0.9 cm to 1.7 ± 0.7 cm ($\bar{X} \pm s_{\bar{X}}$). Thallus height ranged from 1.2 ± 0.4 cm to 0.8 ± 0.2 cm; thallus mass ranged from 2.1 ± 2 g to 0.3 ± 0.03 g. These three morphological variables were highest during November, January and February and lowest in September (Table 1).

The medullar cell layers had hyaline cells of differing size and shape, according to the subcortical section approximation. In this layer, it was possible to identify medullar cells of first, second and third order. The third-order medullar cells, located in both the lower and middle layers, were irregularly shaped with anastomoses (Figs 5, 6). The length and width of these medullar cells were significantly different between months (Table 1). The longer cells were 70–72 ± 8 μm and the shortest were 57 ± 12 μm. The greatest width was 42.2 ± 6.7 μm and the lowest 38 ± 7.5 μm. For these two variables, the maximum values were reported in February, March and November and the minimum in December (Table 1).

The outermost medullary layer had oblong cells with dichotomous branching, containing first- and second-order cells (Fig. 7). The length of these cells was significantly different between months, but the width was similar over the months (Table 1). The greatest length of first-order cells was reported in November, with 32 ± 3 μm, and the shortest in December and March, with 27 ± 6 μm. The greatest length of second-order cells was in November, with 49 ± 4.7 μm, and the shortest in December, with 38.2 ± 6.7 μm. The width of the first order was 19.5 ± 1 μm and of the second order was 30.3 ± 1.3 μm (Table 1).

The subcortical layer contained undifferentiated smaller cells, with adhering assimilating filaments. The subcortical cell length was significantly different between months, but the width was over the months (Table 1). The greatest length was reported in February, with 17.3 ± 2.5 μm, and shortest in March and April, with 13.5 ± 2 and 13.3 ± 1.5 μm, respectively. The width of these cells was 10.8 ± 0.4 μm.

The cortical assimilatory filaments had intercalary unilocular and plurilocular sporangia (Figs 7–9). The assimilatory filaments were three to four cells long and arranged singly or in groups of up to three. The terminal cell was ovoid to globose and contained numerous phaeoplasts (Figs 7, 9). The assimilating filament length was significantly different among months; they were longest in January and February (42.5 ± 1.5 μm and 44.8 ± 8.7 μm, respectively), and shorter (33.3 ± 4.2 μm) in the remaining months (Table 1). The width varied between 7.3 ± 0.5 μm and 10.2 ± 2.5 μm; the widest occurred in December, January, February and November, and the narrowest in April (Table 1).

The hyaline hairs were scattered and solitarily on the surface of the macrothalli (Fig. 8); they originated from the outermost medullary cells, and were 8 ± 1.5 μm wide (Table 1).

The plurilocular sporangia on the macrothalli of *Leathesia marina* were born as singles to triplets from subcortical cells (Fig. 7). The length varied significantly among months, but the width was homogeneous (Table 1). The greatest length was found in April, with 35.0 ± 11.5 μm, and the shortest in November, with 24.3 ± 3.5 μm. The width was constant at 6 ± 2 μm. In most thalli, the uniseriate sporangia were 5–9 ± 1 loculi. Occasionally, biseriate sporangia were recorded, having 12–13 ± 2.1 loculi (Fig. 10).

The unilocular sporangia were ovoid and sessile, and grew from the basal cells of assimilating filaments (Figs 11 and 12). The size of the unilocular sporangia changed over time (Table

←

Fig. 6. Detail of third-order medullary cell of macrothallus in transverse section. Scale bar = 10 μm.
Fig. 7. Medullary cells of first- (1°) and second-order (2°) assimilating filaments (white arrow) and uniseriate plurilocular sporangia (black arrow) with numerous loculi. Scale bar = 10 μm.
Fig. 8. SEM photograph showing detail of the cortical layer with hyaline hairs. Scale bar = 20 μm.
Fig. 9. SEM photograph of assimilating filaments in lateral view. Scale bar = 20 μm.
Fig. 10. Plurilocular sporangia with two or more rows of loculi. Scale bar = 30 μm.
Fig. 11. Unilocular sporangium on basal cell of assimilatory filament. Scale bar = 30 μm.
Fig. 12. Detail of unilocular sporangium showing numerous spores inside. Scale bar = 10 μm.
Fig. 13. SEM photograph of filaments of microthalli in culture conditions. Scale bar = 6 μm.
Fig. 14. Lateral filament of microthallus in nature bearing plurilocular gametangium with a single row of loculi. Scale bar = 10 μm.

Table 1. *Leathesia marina*. Effects of months on vegetative and reproductive structures. Model I ANOVA. Samples collected from September to April; df, degrees of freedom; MS, mean square; F, ANOVA statistic; *P*, probability. Significant differences in both macrothalli and microthalli and sampled months ($\alpha < 0.05$) are indicated by different letters using Tukey's honestly significant difference (HSD). Months' abbreviations: J, January; F, February; M, March; A, April; S, September; O, October; N, November; D, December.

Parameter	df	MS	*F*	*P*	HSD
In macrothallus samples					
Maximum diameter (cm)	7	3.976	8.37	< 0.0001	(a)S, (ab)D-M-A, (ac)N, (c)J-F
Thallus height (cm)	7	0.338	2.65	0.014	(a)S, (ab)D-O, (ac)J-M-A-N, (c)F
Drained weight mass (g)	7	6.953	6.03	< 0.0001	(a)S, (ab)A, (b)D-M-O, (bc)J, (c)F-N
Medullar cells: first order					
length	7	0.110	3.95	< 0.0001	(ab)D-M, (ac)F-S, (b)A, (bc)J-O, (c)N
width	7	0.032	0.78	0.60	—
Medullar cells: second order					
length	7	0.320	5.70	< 0.0001	(a) D, (ab)J-A, (ac)S, (bc)F-M-O, (c)N
width	7	0.061	1.35	0.23	—
Medullar cells: third order					
length	7	0.750	6.02	< 0.0001	(a)D, (ab)J, (ac)S-O, (bc)A, (c)F-M-N
width	7	0.350	5.37	< 0.0001	(a)D, (ab)S, (ac)M-O, (bc)J-A-N, (c)F
Subcortical cells					
length	7	0.056	4.70	< 0.0001	(a)M-A, (ab)D-S, (ac)O-N, (bc)J, (c)F
width	7	0.003	0.88	0.52	—
Assimilator filaments					
length	7	0.726	9.00	< 0.0001	(a)D-M-A-O-N,)ab)S, (b)J-F
width	7	0.065	11.43	< 0.0001	(a)A, (ab)M, (ac)O-S, (c)D-J-F-N
cells (no.)	7	0.982	3.33	0.0026	(a)N, (ab)D-F-M-S-O, (b)J
Hairs					
width	7	0.006	1.31	0.25	—
Unilocular sporangia					
length	7	0.927	9.60	< 0.0001	(a)D-A-O-N, (ab)S, (ac)M, (b)J, (bc)F
width	7	0.050	3.72	0.0011	(a)D-A, (ab)M-S-O-N, (b)J-F
number	7	431.370	8.04	< 0.0001	(a)D, (ab)J-F-N, (b)M-O, (abc)S, c)A
Plurilocular sporangia					
length	7	0.323	3.18	0.0041	(a)N, (ab)D-J-F-M-S, (b)A
width	7	0.041	1.74	0.11	—
number	7	2147.800	12.90	< 0.0001	(a)F-M, (ab)J, (b)D-N, (abc)A-S, (c)O
uniseriate loculi (no.)	7	16.538	10.32	< 0.0001	(a)S, (ab)N-O, (ac)J-F-A, (c)M
biseriate loculi (no.)	5	1.590	0.40	0.84	—
Cover (%)	7	218.650	18.55	< 0.0001	(a)F-M-A-S-O-N, (b)D-J
Number of individuals/m^2	7	2230.310	15.01	< 0.0001	(a)A-S-O, (ab)M-N, (ac)F, (c)D-J
In microthallus samples					
Cell's prostrate filament					
length	3	0.04	2.6	0.05	—
width	3	0.01	2.07	0.11	—
Plurilocular sporangia					
length	3	0.24	2.11	0.1	—
width	3	0.041	4.01	0.01	(a)M, (ab)A-O, (b)S
number	3	36.09	0.88	0.45	-
biseriate loculi (no.)	3	89.69	9.52	0.0001	(a)M-A-O, (b)S
Abundance	3	0.750	4.16	0.008	(a)S, (ab)M-O, (b)A

1). Larger unilocular sporangia were observed in January and February; the maximum length and width were 35.2 ± 10.7 μm and 16.8 ± 2.5 μm, respectively. The minimum length was 20.0 ± 4.5 μm, recorded in December, April, October and November, and the minimum width was 12.4 ± 4.7 μm, recorded in December and April.

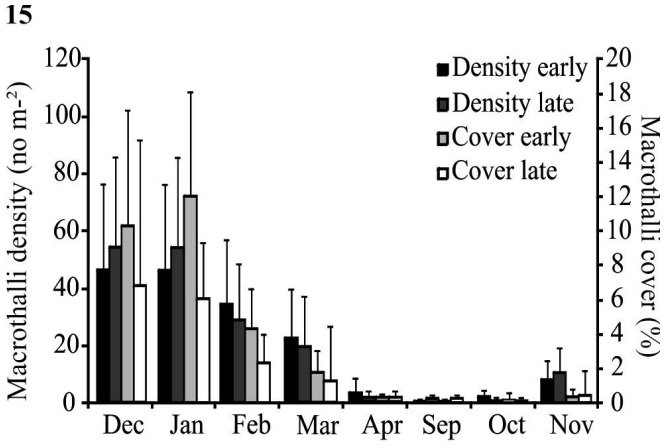
Microthalli of *Leathesia marina*

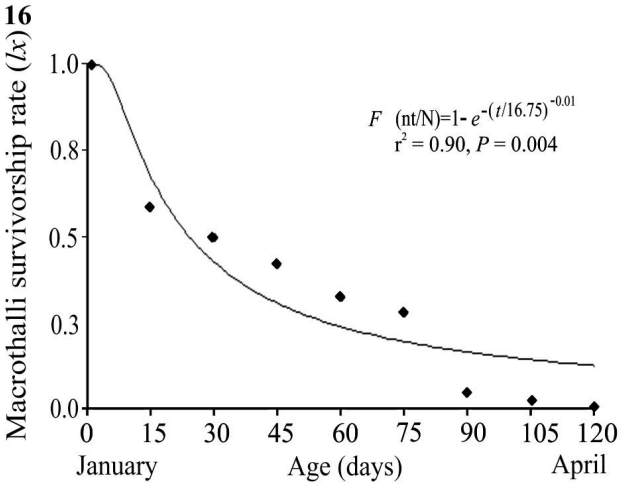
Microthalli associated with macrothalli of *L. marina* were found in March–April and September–October. These microthalli consisted of prostrate filaments of rectangular cells, with lateral branches of irregular arrangement (Fig. 13). The rectangular cells had a single phaeoplast. Cells were 14.2 ± 2.2 μm long and 4.7 ± 0.9 μm wide. These dimensions were homogeneous over different sampling times (months) (Table 1).

On the microthalli, terminal and intercalary plurilocular gametangia were formed individually from rectangular cells. These gametangia consisted of one or two rows of loculi (Fig. 14). Gametangial length was homogeneous in different samples, reaching 16.7 ± 4.1 μm, but there were significant differences in width. The greatest width was in March (13.8 ± 2 μm) and the lowest was in September (10.5 ± 2.5 μm). The number of loculi/gametangium varied between months, being highest in September (17.2 ± 4 loculi/gametangium) and lowest in March, April and October (11.6 ± 2 loculi/gametangium; Table 1).

Macrothalli and microthalli of *Leathesia marina* in nature

The cover and density of macrothalli of *L. marina* were significantly different from month to month (Table1). The highest cover and density were found in late December and





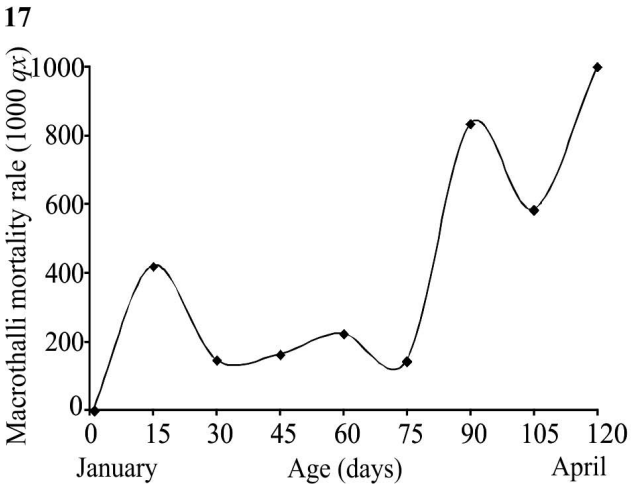


Fig. 15. Macrothallus cover (mean ± standard error of the mean) and density (mean ± standard error of the mean) of *Leathesia marina* estimated over periods of 15 days, from December to November.

Fig. 16. Survivorship curve of macrothalli of *Leathesai marina* (*lx*) fitted to a Weibull.

Fig. 17. Mortality rate of macrothalli of *Leathesia marina* (1000 *qx*) estimated in periods of 15 days, from December to November.

early January, reaching 10.7–12.0% ± 8.5% and 54–69 ± 31 individuals m^{-2}, respectively (Fig. 15). In the remaining months, both the cover and the density decreased. In September cover reached a minimum (0.08% ± 0.1%) and the density was no more than two individuals m^{-2} in April, September and October (Fig. 15).

The recruitment of new individuals was highest in October and November, reaching values of 129% and 133%, respectively. The emergence of new individuals coincided with periods of macrothallus recruitment at the start of the warmer season. At the end of January, a population decline was observed due to both mortality and detachment of thalli, with no new individual recruitment. The survival of recruits of *Leathesia marina* during 105 days of observation from January to April is shown in Fig. 16 and Table 2. The data were fitted to a Weibull curve as:

$$F(nt/N) = 1 - e^{-(t/16.74)^{-0.01}} \qquad (r^2 = 0.90,\ P = 0.004), \quad (2)$$

when N was the number of individuals, nt the number of surviving individuals at time t, and t the age measured in days. In January (0–15 days), a strong decrease in survival was observed, followed by a stable period, with a gradual decrease of recruits during February and March (30–75 days). In March (around day 75) a steep decline in survival was observed, and finally in April the macrothalli of *L. marina* disappeared in the colder season. The survivorship curve was fitted to a type III, indicating high mortality of juvenile and low mortality of adult thalli. Observing the mortality rate (1000 *qx*), two peaks of high mortality were noticed (Fig. 17, Table 2). The first was in January (days 0–15) and coincided with a high percent cover of *L. marina* (Fig. 16), and the second was in late March (day 75). The life table (Table 2) shows that the life expectancy (*ex*) decreased over time.

The reproductive output of macrothalli of *Leathesia marina* was significantly different among months (Table 1). The most unilocular sporangia were in April, reaching 17 ± 7 sporangia/625 μm of thallus examined. Also in this month, the most empty unilocular sporangia number was observed: 13 ± 5.2 empty sporangia/625 μm of thallus. In September, all sporangia had spores (Fig. 18, Table 1). The lowest number of sporangia was in December, when only 1 sporangium/625 μm thallus was observed. Regarding plurilocular sporangia, the highest number was in October, with 33 ± 19 sporangia/625 μm of thallus, and the lowest was in February and March, with 3 ± 3 sporangia/625 μm of thallus (Fig. 19, Table 1).

The abundance of microthalli of *Leathesia marina* was significantly different among months. The highest abundance was in April (0.55 ± 0.5) and the lowest was in September (0.10 ± 0.3). The mean reproductive output of microthalli was 11 ± 6 plurilocular gametangia/625 μm of thallus, and this was homogeneous over different months.

Relationship between parameters measured in *Leathesia marina* and environmental factors

The dynamics and morphology of *L. marina* were related to environmental parameters. The first two axes of the PCA explained 80.7% of the joint variation between the environmental and biological factors (Fig. 20). PC 1 was mainly related to the seasonal variability of the samples. Samples

Table 2. Life table. *nx*, survivor number at beginning of day *x*; *lx*, proportion of the original cohort that survives until day *x*; *dx*, number dying in day *x*; 1000 *qx*, number dying *per* 1000 alive at beginning of day *x*; *Lx*, number of thalli alive between day *x* and day *x* + 1; *Tx*, sum of weeks of life remaining to those aged and *ex*, average life expectancy (days) of those aged *x*.

Days	*nx*	*lx*	*dx*	1000 *qx*	*Lx*	*Tx*	*ex*
0	260	1.00	109	419.2	205.5	691	2.66
15	151	0.58	22	145.7	140	485.5	3.22
30	129	0.50	21	162.8	118.5	345.5	2.68
45	108	0.42	24	222.2	96	227	2.10
60	84	0.32	12	142.8	78	131	1.56
75	72	0.28	60	833.3	42	53	0.74
90	12	0.05	7	583.3	8.5	11	0.92
105	5	0.02	0	1000	2.5	2.5	0.50

from warmer months (November–February) were grouped on the more negative side of PCA axis 1, whereas samples from the temperate periods (March–April and September–October) were on the more positive side of PCA axis 1. The left side of PC 1 (November–February) was characterized by high density, cover and size of macrothalli, jointly with high seawater temperatures, long days and high radiation. Macrothallus density and cover were positively correlated with radiation and day length (Spearman rank correlation, cover–radiation: $N = 96$, rs = 0.82, $P = 0.014$; cover–daylength: rs = 0.74, $P = 0.035$; density-radiation: rs = 0.70, $P = 0.045$; density–day length: rs = 0.74, $P = 0.034$). Macrothallus mass and height were positively correlated with radiation (rs = 0.74, $P = 0.033$) and seawater temperature (rs = 0.76, $P = 0.031$), respectively. These correlations indicate that vegetative growth of *L. marina* was favoured by summer conditions.

The right side of PC 1 (March–April and September–October) was characterized by high abundance and reproductive output of microthalli, high number of sporangia in macrothalli and high number of gametangia in the microthalli,

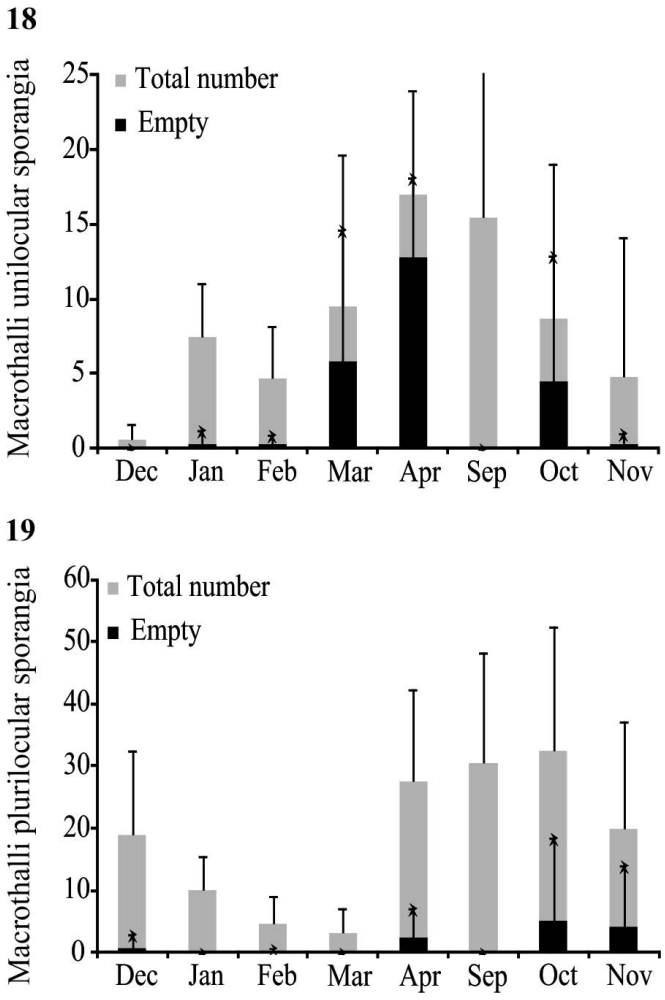


Fig. 18. Reproductive output of macrothalli. Unilocular sporangia and empty sporangia (mean ± standard error of the mean) numbers estimated from December to November (625 μm of thallus examined).

Fig. 19. Reproductive output of macrothalli. Plurilocular sporangia and empty sporangia (mean ± standard error of the mean) numbers estimated from December to November (625 μm thallus examined).

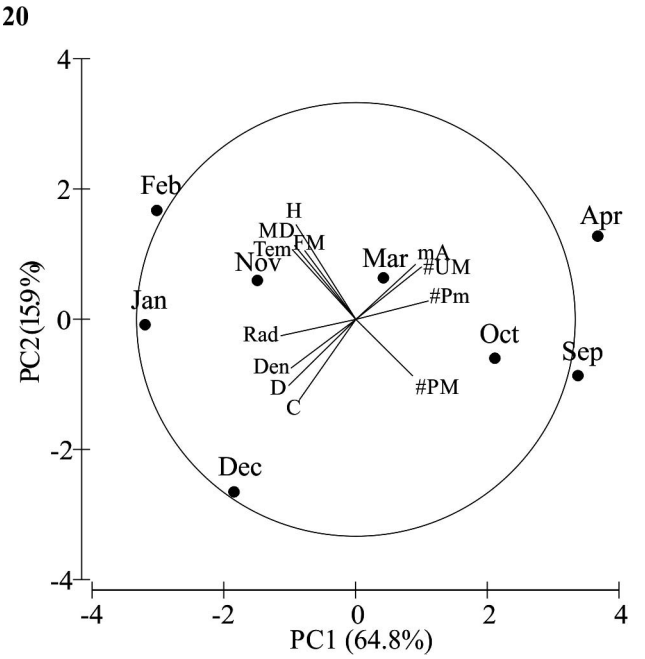


Fig. 20. PCA biplot, showing temporal relationship between environmental parameters [seawater temperature (Tem), day length (D) radiation (Rad)] and parameters measured in *Leathesia marina* [macrothallus diameter (MD), macrothallus fresh mass (FM), macrothallus height (H), plurilocular sporangia number on macrothalli (#PM), unilocular sporangia number on macrothalli (#UM), macrothallus density (Den), microthallus cover (CM), microthallus abundance (mA), plurilocular gametangia number on microthalli (#Pm)].

jointly with low seawater temperatures, day length and radiation. Microthallus abundance was negatively correlated with radiation and day length (Spearman rank correlation: $N = 96$, rs = –0.82, $P = 0.02$; rs = –0.83, $P = 0.01$, respectively). The number of unilocular sporangia in macrothalli and plurilocular gametangia in microthalli were negatively correlated with radiation, day length and seawater temperature (sporangia–radiation: rs = –0.85, $P = 0.0076$; sporangia–daylength: rs = –0.93, $P < 0.0001$; rs = –0.82, $P = 0.01$; rs = –0.83, $P < 0.01$; gametangia–radiation: rs = –0.75, $P = 0.033$; gametangia–seawater temperature: rs = –0.88, $P = 0.0043$). Therefore the development of reproductive structures was associated with winter conditions, that is, situations that were not optimal for vegetative growth of macrothalli. This result was also observed in the negative correlation between macrothallus density and number of unilocular and plurilocular sporangia (density–unilocular: rs = –0.74, $P = 0.032$, density–plurilocular: rs = –0.71, $P = 0.045$), indicating that as macrothallus density decreases, the number of both types of sporangia increases.

DISCUSSION

The population of *Leathesia marina* from the northern Patagonian coast was distributed mainly in the low intertidal zone. The macrothalli were epiphytic on *Corallina officinalis* and mussels, e.g. *Brachidontes rodriguezii* and *Perumytius purpuratus,* and they were never found on bare substrate. In previous studies carried out in this area and along the southern coast of Argentina, macrothalli of *L. marina* have also been observed as epiphytes on beds of *C. officinalis* and on mussels (Kühnemann 1969; Quartino & Boraso de Zaixso 1996). Also, on the southern coast of California, *L. marina* was found associated with *Corallina* sp. beds, indicating that this association is essential to the settlement and development of a globose structure (Oates 1989). In addition, the coralline algal turfs, *C. officinalis,* with strong adherence to rocky substrate, often trap considerable quantities of sediment and retain large amounts of water during low tides (Akioka *et al.* 1999), producing a favourable habitat for the growth of *L. marina*.

The presence of macrothalli of *L. marina* in Nova Scotia, Canada was observed only from June to August (Chapman & Goudey 1983). On the Patagonian coast, the occurrence of the macroscopic phase was more extensive, where it was found over 8 months corresponding to the warm seasons. Moreover, microscopic filamentous microthalli were found over 4 months (March–April and September–October). Here, a temporal overlap was observed between the microscopic and macroscopic phases. Even more, the highest reproductive output, with a great increase in the number of sporangia and release of spores, was observed in the macroscopic phase, coincidentally during months where microthalli were found during fall conditions with low seawater temperatures, day length and radiation. Consequently, the occurrence of the microscopic stage might persist during colder months (May–August). Despite the fact that microthalli have not been found in nature during the winter months, it is known that the microscopic stages are critical for the annual dynamics of species.

The seaweeds that manage to colonize the lower intertidal zone seem to be favoured, since they are less exposed to the effects of desiccation and high temperatures, even though they are exposed to waves (Bertness *et al.* 2006). For this reason, high temperatures do not seem to affect the recruitment of macrothalli in *Leathesia marina*, since greater density and cover were recorded in the hottest months. In previous studies, Chapman & Goudey (1983) and Quartino & Boraso de Zaixso (1996) observed higher density of *L. marina* during the hottest months. The irregular cushion-shaped morphology with numerous interstices, together with the longer assimilatory filaments observed during the warm months, could favour water retention and thus avoid desiccation. This phenomenon was also observed in other brown algae with similar thallus shape, i.e. saccate thalli as in *Colpomenia* spp. (Oates 1985).

Aspects of vegetative and reproductive morphology of the macrothalli are within the limits observed by other authors (Okamura 1936; Takamatsu 1939; Abbott & Hollenberg 1976; Womersley 1987; Tanaka *et al.* 2010; Boraso 2013). However, in previous descriptions of this species, the biseriate plurilocular sporangia were never mentioned. Here, the sporangia were found sporadically throughout the year and they were similar to the plurilocular gametangia found on microthalli.

The population dynamics of *Leathesia marina* could be explained as a response to the heteromorphic life cycle, where each phase presented particular adaptations to environmental parameters (Lubchenco & Cubit 1980; Zupan & West 1990; Cunningham *et al.* 1993). The demographic study conducted on *L. marina* by Chapman and Goudey (1983) showed that the dynamics of the macrothalli were regulated by thallus crowding and hence mortality rates increased throughout life. In previous studies by Quartino & Boraso de Zaixso (1996) in Patagonia, overcrowding was not observed and they attributed the disappearance of macrothalli to senescence during the cold season. In this study, a first population decline was observed when *L. marina* reached its maximum cover. Subsequently, the survival rate continued to decline. At the beginning of the colder months, another important population decline was observed, coincident with the end of the macroscopic phase, and with the initiation of the microscopic phase. In this context, our population of *L. marina* seemed to be regulated mainly by seasonal variations, rather than purely demographic responses.

The type III survivorship curves found for macrothalli of *Leathesia marina* have also been reported for other brown algae, such as *Cystoseira osmundacea* (Turner) C.Agardh, *Laminaria farlowii* Setchell and *Macrocystis pyrifera* (Linnaeus) C.Agardh (Dayton *et al.* 1984). This phenomenon was frequent in kelp and other algae that have microscopic phases, which can grow quickly with a massive recruitment followed by high mortality. The mortality rate subsequently declines among the recruits that do ultimately persist (De Wreede & Klinger 1988). However, a study conducted by Chapman & Goudey (1983) on *L. marina* from Nova Scotia showed a type I survivorship curve, characterized by both low prereproductive mortality and high postreproductive mortality. The difference between the populations of *L.*

marina from Patagonia and Nova Scotia is due to the extreme conditions present in Nova Scotia, where the seawater temperature was below 5°C in the winter and above 12°C from June to September (Wilson *et al*. 2015). At Nuevo Gulf the environmental conditions are clearly different, as in this region the highest seawater temperature, radiation and longer days are dominant.

High reproductive output of the macroscopic phase was observed throughout the whole occurrence period in nature. A similar phenomenon was observed for the brown alga *Ralfsia californica* Setchell & N.L.Gardner from Washington (Dethier 1981). Moreover, the high reproductive output of the macroscopic phase could be explained as a response to differential susceptibility to desiccation between the life-cycle stages (Thornber 2006). The prostrate microscopic phase might provide greater protection and stability in the environment (Cunningham *et al*. 1993).

ACKNOWLEDGEMENTS

A.M.P. is a doctoral fellow of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). M.C.G. and E.R.P. are researchers of CONICET and J.F.E. is an associate technician of Centro Nacional Patagónico (CENPAT) Support was provided by grants from the Secretaría de Ciencia y Tecnología de la Universidad Nacional del Sur (PGI CSU-24B/234) and CONICET (PIP-11220130100070CO). We also thank Rosemary Scoffield, MSc and Dr M. Emilia Croce for reading the manuscript.

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Received 20 October 2016; accepted 21 April 2017
Associate Editor: Ricardo A. Scrosati