Spatial and temporal variation in algal epiphyte distribution on *Ulva* sp. (Ulvales, Chlorophyta) from northern Patagonia in Argentina

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ABSTRACT: In this study of northern Patagonia in Argentina, we examined the annual cycle of the epiphyte composition and abundance on *Ulva* sp. with respect to intertidal elevation, season and position on host. We found spatial segregation in most environmental gradients. Local environmental conditions, intertidal levels, seasons and portion of host thalli had specific patterns of algal epiphytic community. The host population exhibited seasonality and differential morphological attributes according to the intertidal elevation. Thalli of *Ulva* sp. had the highest maximum diameters and were heavier in spring and summer at the upper elevation. Epiphytes were more abundant growing on host thalli at middle elevations. Epiphytic Dinophyceae, Chlorophyceae and Cyanophyceae presented clear seasonality. Epiphyte abundances were related to periods with high seawater temperature, long days and high radiation. *Lyngbya* sp., *Cocconeis* sp., *Navicula* spp., *Rhabdonema arcuatum*, *Stylonema alsidii* and *Myrionema strangulans* had the highest frequency. The highest epiphyte abundance was observed in holdfasts, with *M. strangulans* having the highest frequency and cover. These results are relevant to commercial aquaculture ventures for food and feed sources and application in bioremediation.

KEY WORDS: Dynamic and zonation gradients, Epiphytic assemblage, *Myrionema strangulans*, North Patagonian Atlantic coast, *Ulva* sp. seasonality

INTRODUCTION

For decades, species of the macroalgal genus *Ulva* (Chlorophyta, Ulvales) have been used as biomass sources for food and feed purposes due to their high contents of vitamins, trace metals and dietary fibers (Lahaye & Jegou 1993; Ohno 1993; Bolton *et al*. 2009; Taboada *et al*. 2010). Currently, global warming issues and the limited fossil fuels supply have drawn attention to algae as an energy crop as well. Therefore, *Ulva* spp. are cultivated in many parts of the world in pilot commercial systems (De Busk *et al*. 1986; Israel *et al*. 1995; Neori *et al*. 2000, 2003; Bruhn *et al*. 2011). Because of their high growth potential, *Ulva* species are capable of high rates of nutrient assimilation, especially ammonium, and good growth in eutrophic waters, qualifying this genus for bioremediation (Gaevert *et al*. 2007; Bolton *et al*. 2009) and aquaculture applications (McKendry 2002; Seppälä *et al*. 2008).

Epiphytism on marine benthic macroalgae and seagrass communities is a widespread phenomenon. Algal epiphytes may use the host for acquiring enhanced proximity to light and dissolved nutrients as well as a substrate for attachment (Harlin 1980). Consequently, competition for space is intense

(Rindi & Guiry 2004), and the epiphytes may normally cause negative effects on host thalli, such as decreasing their performance (Buschmann & Gómez 1993), growth and reproduction rates (Kraberg & Norton 2007) and increasing the drag effect with subsequent breakage and detachment of thalli (Ruesink 1998; Anderson & Martone 2014). However, epiphytes also have beneficial effects on hosts, such as providing food and habitat for animals (Viejo & Åberg 2003) and protecting hosts against the effects of both desiccation and excess light at low tides (Richardson 1980).

Previous studies showed that epiphyte community structure can be influenced by biotic factors, such as host attributes and grazing pressure by herbivores (Mabrouk *et al*. 2011), as well as by abiotic factors, such as light, temperature, nutrients and water motion (Lavery *et al*. 2007; Mabrouk *et al*. 2012). Also, there is a relationship between epiphyte composition and abundance and the algal host surface features (Longtin *et al*. 2009). As a consequence, it is important to evaluate epiphyte distribution in conjunction with their hosts and other primary-space holders since different macroalgae do not support algal epiphytes in the same way. This differentiation is related to factors such as algal architecture, the possession of chemical defenses and/or types of life cycles (Michael *et al*. 2008) as well environmental gradients that help predict species distributions. Physical environmental factors, such as photoperiod (Hanelt & Roleda 2009), wave exposure (Kendrick & Burt 1997),

seawater temperature and nutrient supply, are highly variable on marine shores, and each gradient might help to identify the major sources of variation affecting epiphyte distribution.

Along the intertidal gradient, physiological stresses related to irradiance, temperature, desiccation and osmotic potential increase vertically with elevation because of tidal dynamics (Raffaelli & Hawkins 1996; Garbary 2007). As a result, vertical gradients of environmental stress are major factors affecting the distribution of organisms across elevations (Menge & Branch 2001; Bruno *et al.* 2003). Longtin *et al.* (2009) suggested that the variability of these factors might explain variation and distribution in epiphyte communities.

Here we test the notion that environmental gradients across intertidal elevations, seasons and host fronds explain the variation in abundance of epiphytes on *Ulva* sp. on the shores of Argentine Patagonia. This variability of epiphyte distribution can be predicted since different epiphytic taxa experience different ecological conditions. Our first aim was to evaluate intertidal elevation and season across populations of *Ulva* sp. with respect to epiphytic assemblage. According to previous observations in other host macro-algae, we hypothesized that *Ulva* sp. and its epiphytes can be characterised with respect to abundance, richness and diversity across intertidal elevations, seasons and host regions.

Myrionema strangulans Greville (Ectocarpales, Phaeophyceae) and other epiphytic biota are commonly observed in populations of *Ulva* (Bolton *et al.* 2009; Siniscalchi *et al.* 2012). In Argentine populations, *M. strangulans* had an epiphyte frequency near 100%, where it could result in perforations on host cuticles, massive depigmentation, cellular disorganization, and subsequent thallus rupture (Siniscalchi *et al.* 2012). At this high frequency, it could be a potential problem for future local aquaculture enterprises. Hence, our second objective was to test the effects of intertidal elevations and seasons on the cover of *M. strangulans* growing in different portions of the host thalli. A third objective was to relate the environmental parameters of vegetative host attributes and epiphyte communities to the abundance of *M. strangulans*.

Thus, we addressed the following questions: (1) Does epiphyte abundance change across intertidal elevations, seasons and host parts of thalli of *Ulva* sp. in Patagonia? (2) How does *M. strangulans* cover vary with these three factors? (3) What are the main species assemblages characterising each intertidal level, season and host region? (4) Is there any vegetative host phenology variation across intertidal elevations? (5) Is there any correlation between the environmental parameters, epiphytism and vegetative host phenology?

Note on *Ulva* taxonomy

Species of *Ulva* are difficult to identify due to intraspecific variability in the rather few morphological and anatomical characters used for species discrimination (Hoesksema & van den Hoek 1983; Koeman 1985). Moreover, many of these characters vary with the environmental conditions associated with geographical location, seasonality, habitat and age (Phillips 1988; Woolcott & King 1993). Along the shores of

Patagonian Argentina, *Ulva* is well represented with populations of *Ulva californica* Wille; *Ulva compressa* L.; *Ulva fasciata* (Roth) Martinius; *Ulva flexuosa* Wulfen; *Ulva hookeriana* (Kützing) Hayden, Maggs, Silva, Stanhope & Waaland; *Ulva intestinalis* Linnaeus; *Ulva lactuca* Linnaeus; *Ulva linza* Linnaeus; *Ulva prolifera* O.F.Müller; and *Ulva rigida* (C.Agardh) Thuret (Boraso de Zaixso 2013; Guiry & Guiry 2015).

Since the beginning of the 21st century, *Ulva* taxonomy has been in a major upheaval (Hayden *et al.* 2003). DNA-based studies have revealed evident discrepancies between morphospecies and actual taxonomic entities (O'Kelly *et al.* 2010). In particular, the name '*Ulva lactuca*' has currently been applied to many different species of *Ulva*, and even the most commonly accepted DNA-based concept of this species is in error because it does not match with the DNA signature of the holotype specimen of *U. lactuca* (O'Kelly *et al.* 2010). Due to the absence of DNA-based studies on the *Ulva* species present along the Patagonian coast of South America, many of the names of European species may have been misapplied to Patagonian entities. We consider it inappropriate to apply any more precise name than '*Ulva* sp.' to the individuals examined in this study, all of which were flattened and foliose.

MATERIAL AND METHODS

This study was performed on the northern Patagonian coast of Argentina on the Golfo Nuevo (42°78′S, 64°95′W) (Fig. 1). Golfo Nuevo is a semienclosed basin of low hydrodynamics, located in the transition zone between cold-temperate and warm-temperate biogeographic regions of the southwestern Atlantic Ocean. The region is characterised by extreme weather conditions, with a predominance of strong winds and low humidity from the west (Paruelo *et al.* 1998). These strong, dry winds, combined with low local rainfall, make the Patagonian intertidal zone the place with the highest desiccation stress recorded for rocky shore communities (Bertness *et al.* 2006). The tide regime is semidiurnal, and the mean amplitude is 3.8 m, reaching 5.7 m in spring. Water temperature and salinity fluctuate yearly between 10°C and 19.5°C and 33.7‰ and 33.9‰, respectively (Meteocean-Cenpat-Conicet 2015).

Punta Este intertidal shores consist of a siltstone platform partially covered by unconsolidated sand and gravel. In these coastal zones, the macroalgal communities are composed of small or medium-size species (smaller than 20 cm high) of different morphological types, including filamentous, foliose, crustose and turf forming algae. Animal benthic communities in this area are dominated by *Brachidontes rodriguezii* (d'Orbigny 1842) and *Perumytilus purpuratus* (Lamarck 1819) (Kelaher *et al.* 2007).

During sampling, three horizontal levels in the intertidal area were identified. The upper intertidal level (UL) presented a mean height of 4.03 m above sea level, the mid-intertidal level (ML) 2.81 m above sea level and the low-intertidal level (LL) 2.05 m above sea level (Fig. 2).

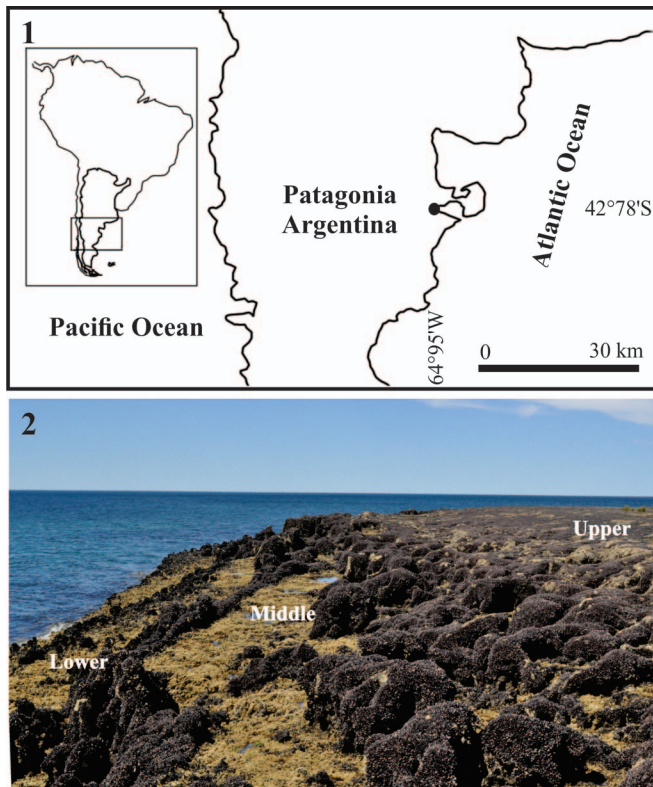Distribution patterns of benthic invertebrates and algae were defined based on the topography that determined

Fig. 1. Sampling site on Golfo Nuevo on the northern shore of the Atlantic Patagonian coast.
Fig. 2. Sampling site indicating the intertidal levels: lower, middle and high.

different types of water exposure. The upper level was defined as the one where mussels and barnacles were abundant and where desiccation was highest during low tides. The middle level was defined as being covered by a compact mussel bed, and the lower level was dominated by dense coralline algal cover. The upper intertidal level had an average exposure time of 4.03 h, while the middle and the lower level exposures were 2.81 h and 2.05 h, respectively. Surface seawater temperatures and day length were measured daily at the sampling location. Radiation data were provided by Automatic Meteorological Station of Climatology Laboratory of CENPAT-CONICET. Surface seawater temperatures and day length were measured daily at the sampling location.

Fronds of *Ulva* sp. were collected monthly from the three intertidal elevations (UL, ML and LL) from January to December 2014. Each month, 20 complete attached individuals were collected randomly from each level. All specimens were squeezed by hand to remove excess seawater and subsequently transported to the laboratory in closed plastic bags. Samples were stored overnight at 5°C. The following day, each specimen was washed thoroughly with seawater to remove adhering sand. Maximum diameter and wet weight were determined monthly in 20 individuals from each intertidal level.

In each specimen from each intertidal elevation, 10 × 10-mm areas were randomly selected from periphery, middle and holdfast regions of thalli. Each sample from each frond

were stored in FAA (ethyl alcohol:formaldehyde:acetic acid at 8:1:1) such that each portion of host frond was maintained as a separate unit.

Epiphytes were identified and counted using a Nikon Eclipse 1 TE 300 microscope (Tokyo, Japan) equipped with a Nikon FDX 35 camera. Counting was normally performed at ×400 magnification. For filamentous Cyanophyceae, a ×100 oil immersion objective was used. Cyanobacterial filaments and nonfilamentous colonies, brown algae and single-celled green algae were counted as individuals. Four groups of epiphytes were recognized for recording epiphyte incidence: (1) very frequent (present on more than 150 thalli), (2) frequent (75–149 thalli), (3) rare (15–74 thalli) and (4), occasional (fewer than 14 thalli).

Host thallus fragments were fixed in 0.01 M sodium cacodilate (pH 7.2) buffer containing 2.5% glutaraldehyde at 5°C for 2 h. They were subsequently mounted on slides covered with 0.5% poly-D-lysine and dehydrated in a graded acetone series following the protocol of Cáceres (1995). Finally, the samples were critical-point dried for 1 h, coated with gold and observed with a Leo Evo 40 (Jena, Germany) scanning electron microscope (SEM).

Two variables – maximum diameter and wet mass of individual *Ulva* thalli from the intertidal levels and seasons – were evaluated using one-way analysis of variance based on 9999 permutations (PERMANOVA). These tests were made in a resemblance matrix using Euclidean distance with normalized data.

Abundance, expressed as the number of individuals of each epiphyte taxon recorded, was used to describe the epiphyte distribution patterns on fronds. Percent cover was used as an abundance parameter for *M. strangulans*. These parameters were evaluated according to the intertidal levels, seasons (months) and host thallus regions (periphery, middle and holdfast). The different seasons were defined by the following months: autumn (March, April and May), winter (June, July and August), spring (September, October and November) and summer (December, January and February).

Differences between algal epiphyte classes, total species (S), Margalef diversity (d), total load epiphyte abundance and abundance of the most abundant epiphyte (expressed as individual number/mm^2 host) were tested by one-way analysis of similarities test (ANOSIM) when gradients between intertidal levels and host thalli regions were evaluated. In seasonality comparisons, ANOSIM two-way nested [seasons (months)] with 9999 permutations tests were used. These tests were performed using a Bray–Curtis similarity matrix applying the square-root and log $(x + 1)$ data transformations. In each ANOSIM test, the null hypothesis that there were no significant differences between groups was rejected if the significance level (P) was < 0.05 (groups for host thalli regions: periphery, middle section, holdfast; groups for seasonal comparison: autumn, winter, spring and summer; and groups for intertidal level: upper, middle and lower). When significant differences were detected between *a priori* groups, the R statistic was used to determine the extent of those differences. Similarity percentages (SIMPER) were used to explain which epiphyte taxa were in each group and to distinguish between each pair

of groups according to the gradients in the intertidal levels, seasons and host thallus regions.

Biological data were related to environmental factors by means of principal component analyses (PCA). The biological variables considered in the multivariate analysis were the following: (Hmd), host maximum diameter (cm); (Hwm), host wet mass (g); (E Abun), total epiphyte abundance (number of individuals); and (My cov), *M. strangulans* cover (mm^2/host tissue). The abiotic factors considered in the PCA were (T), seawater temperature (°C); (D), length of daily light period; and (R), solar radiation (W/m^2).

The distribution gradients and epiphyte assemblage compositions were tested using the multivariate data analysis PRIMER (Plymouth Routines in Multivariate Ecological Research) statistical package (Clarke & Warwick 2001).

RESULTS

Ulva sp. morphological attributes across elevations and seasons

Host distribution across the intertidal levels was not uniform. Host thalli were observed in the upper and middle levels year-round; whereas, they were absent in the lower level from June to September. Host morphological variables, such as maximum diameter and wet mass, showed spatial variations. Both variables were higher in the upper than in the middle and lower levels (maximum diameter, PERMANOVA, pseudo-F = 22.637, P = 0.0001; wet mass, pseudo-F = 9.0364, P = 0.001). Neither host variable was statistically different between middle and lower elevations (Fig. 3).

Moreover, these variables showed seasonal variation (maximum diameter, PERMANOVA, pseudo-F = 13.899, P = 0.001; wet mass, pseudo-F = 34.772, P = 0.001). The host maximum diameter was higher during spring–summer than in autumn–winter, whereas host thalli had greater mass in summer, decreasing towards autumn (Fig. 4).

Epiphytic algae identified on *Ulva* sp

During this study, 26 epiphytic taxa were found on *Ulva* sp. (Table 1): seven Cyanophyceae, nine Bacillariophyceae (including centric diatoms), two Dinophyceae, three Rhodophyceae, two Chlorophyceae and three Phaeophyceae. The epiphyte incidence rate is shown in Table 1. According to the incidence rate, *Lyngbya* sp., *Cocconeis* sp., *Navicula* sp., *Rhabdonema arcuatum* (Lyngbye) Kützing, *Stylonema alsidii* (Zanardini) Drew and *M. strangulans* Greville are very frequent. In addition, *M. strangulans* had the highest prevalence, infecting more than 280 fronds of *Ulva* sp. *Oscillatoria pulchra* Lindstedt, *Peridinium* sp. and *Ulvella* sp. were frequent taxa, based on incidence, and the remaining epiphytes were regarded as either rare or occasional. On the other hand, when considering the total abundance (expressed as individual number/mm^2 host), eight abundant taxa were identified: *Lyngbya* sp., *Cocconeis* sp., *Navicula* sp., *R. arcuatum*, *S. alsidii*, *O. pulchra*, *Chamaecalyx* sp. and *Merismopedia* sp. Each of these taxa had a higher than 10% of the total abundance.
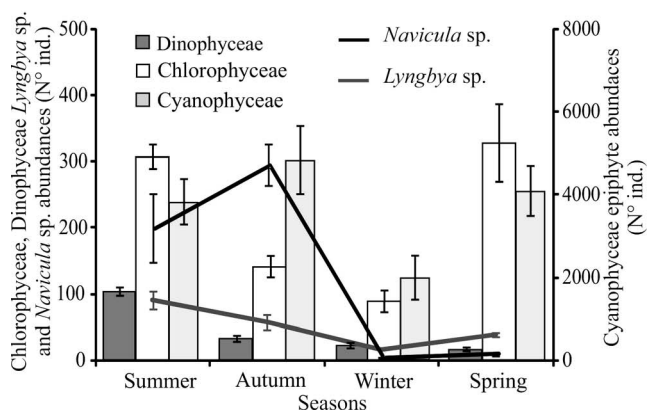
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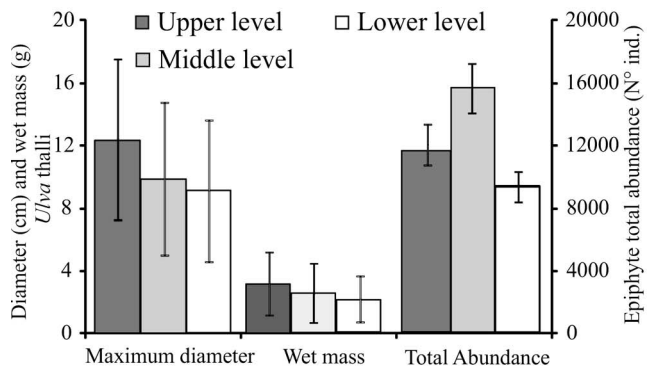
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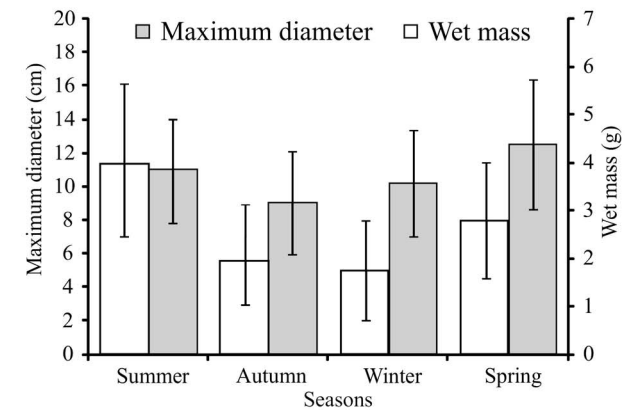
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Fig. 3. *Ulva* sp. attributes: maximum diameter (cm) and wet mass (g) across intertidal elevations. Total epiphyte abundance expressed as the number of individuals across intertidal elevations.
Fig. 4. Seasonal variation in maximum diameter (cm) and wet mass (g) of host *Ulva* thalli.
Fig. 5. Seasonal abundance of algal epiphyte classes: Dinophyceae, Chlorophyceae and Cyanophyceae and epiphyte taxa: *Navicula* sp. and *Lyngbya* sp. (all expressed as number of individuals).

Different epiphyte algal classes had unequal abundance (ANOSIM global R = 0.067, P = 0.001). Cyanophyceae and Bacillariophyceae were significantly more abundant than the remaining algal classes.

Table 1. Algal epiphytes list from December 2013 to November 2014. Epiphyte abundance in different host regions, incidence rate and epiphyte groups are reported. Incidence rate was expressed as % of total thalli epiphytised by each epiphyte taxa.

Epiphytic taxa	Upper, no. epiphytised thalli			Middle, no. epiphytised thalli			Lower, no. epiphytised thalli			Incidence rate (%)	Epiphytic groups
	Periphery	Middle	Holdfast	Periphery	Middle	Holdfast	Periphery	Middle	Holdfast		
Chamaecalyx sp.	—	2	25	—	4	19	—	2	15	18.61	rare
Lyngbya sp.	10	10	56	15	13	45	19	17	24	58.05	very frequent
Merismopedia sp.	2	2	11	1	—	8	1	—	7	8.88	rare
Oscillatoria pulchra	1	3	32	—	2	25	1	5	18	24.16	frequent
Oscillatoria sp.	—	—	5	—	—	2	—	—	2	2.50	occasional
Anabaena sp.	—	—	—	—	—	—	1	—	—	0.27	occasional
Spirulina sp.	—	—	3	—	1	3	2	—	1	2.77	occasional
Peridinium sp.	17	13	9	14	8	13	5	8	9	26.66	frequent
Prorocentrum sp.	—	—	—	—	2	1	—	1	—	1.11	occasional
'Central diatom' group	1	—	4	—	—	1	2	2	—	2.77	occasional
Cocconeis sp.	18	25	53	36	29	53	16	14	29	75.83	very frequent
Gomphonema sp.	—	—	9	3	4	12	4	4	8	12.22	rare
Navicula sp.	11	15	28	21	19	29	17	12	18	47.22	very frequent
Nitzschia spp.	—	3	6	2	1	4	2	—	7	6.94	rare
Pinnularia sp.	—	—	2	—	2	1	2	—	3	2.77	occasional
Pleurosigma sp.	—	—	2	—	2	4	—	—	1	2.50	occasional
Rhadonema arcuatum	9	10	24	19	25	32	14	9	22	45.55	very frequent
Synedra sp.	2	1	—	1	2	1	5	2	4	5.0	rare
Ceramium spp.	—	1	—	—	—	5	—	1	1	2.22	occasional
Polysiphonia spp.	1	6	6	11	9	10	7	9	10	19.16	rare
Stylonema alsidii	1	7	25	10	9	42	12	12	32	41.66	very frequent
Ulothrix sp.	—	—	—	—	1	—	—	—	—	0.27	occasional
Ulvella sp.	5	8	26	11	13	27	7	12	20	41.66	frequent
Ectocarpus sp.	—	—	—	—	—	1	—	2	1	1.11	occasional
Myrionema strangulans	10	15	58	28	29	49	27	30	42	80.0	very frequent
Sphacelaria sp.	—	—	—	—	—	2	—	—	—	0.55	occasional

Epiphyte distribution across intertidal elevations

Total epiphyte abundance differed between the intertidal levels (ANOSIM global $R = 0.403$, $P = 0.001$). Epiphytes were more abundant on thalli growing in the mid-intertidal level than on fronds collected in the lower and upper levels. The abundance at the lower level was 60% of the abundance at middle elevations (Fig. 3).

On the other hand, different epiphyte algal classes presented equitable abundance along the intertidal elevations (ANOSIM Cyanophyceae global $R = 0.002$, $P = 0.393$; Bacillariophyceae global $R = 0.01$, $P = 0.267$; Phaeophyceae global $R = 0.02$, $P = 0.123$; Dinophyceae global $R = -0.011$, $P = 0.755$; Rhodophyceae global $R = 0.027$, $P = 0.064$; Chlorophyceae global $R = 0.013$, $P = 0.0181$), indicating that epiphytes of any class can colonize *Ulva* thalli growing in any of three intertidal elevations.

Among the most abundant species, only two diatoms, *Navicula* sp. and *R. arcuatum*, presented differences in abundance between the intertidal levels (ANOSIM global $R = 0.071$, $P = 0.037$, and ANOSIM global $R = 0.178$, $P = 0.002$, respectively). Both taxa were more abundant in the mid-intertidal level.

SIMPER analysis made it possible to identify characteristic epiphyte taxa assemblages at each elevation. Middle levels differed from the upper ones by greater abundance and/or the presence of assemblage constituted by *Navicula* sp., *Cocconeis* sp., *M. strangulans*, *Ectocarpus* sp., *Chamaecalyx* sp., *Nitzschia* sp., *Merismopedia* sp., *Lyngbya* sp., *R. arcuatum* and *O. pulchra* (Table 2). By comparing both the lower and the upper elevations, *Cocconeis* sp., *M. strangulans*, *Ectocarpus* sp.,

Merismopedia sp., *Chamaecalyx* sp., *Navicula* sp., *R. arcuatum*, *Nitzschia* sp., *Lyngbya* sp., *S. alsidii* and *Ulvella* sp. were the taxa most responsible for dissimilarity. On the other hand, the lowest dissimilarity percentage was determined between the middle and lower elevations. This dissimilarity was given by five epiphyte taxa: *Navicula* sp., *Cocconeis* sp., *M. strangulans*, *Merismopedia* sp. and *Chamaecalyx* sp. (Table 2). Comparing the assemblages, it was possible to detect no coincident species between the two levels, being *O. pulchra* at the middle elevation and *S. alsidii* and *Ulvella* sp. at the lower level.

Epiphyte distribution across seasons

ANOSIM two-way nested [seasons (months)] indicated that total epiphyte abundance, expressed as the number of individuals, did not show any seasonality between samples (global $R = 0.002$, $P = 0.0433$). Only cyanobacterial, dinophycean and green algal epiphytes showed seasonality (ANOSIM: global $R = 0.076$, $P = 0.002$; global $R = 0.053$, $P = 0.012$; global $R = 0.085$, $P = 0.002$, respectively) (Fig. 5). Dinoflagellates were more abundant in summer and green epiphytes in spring–summer; whereas, the Cyanobacteria were less abundant in winter. Seasonality was observed in *Lyngbya* sp. and *Navicula* sp. (ANOSIM global $R = 0.077$, $P = 0.032$, and ANOSIM global $R = 0.225$, $P = 0.001$, respectively). Both taxa were more abundant in the summer–autumn (Fig. 5).

By observing by each pair of seasons, it was noted that the species assemblages were constituted by common taxa such as *Cocconeis* sp., *M. strangulans*, *Merismopedia* sp., *Chamaecalyx* sp., *Ectocarpus* sp. and *Lyngbya* sp.

Table 2. ANOSIM pairwise tests and similarity of percentage (SIMPER) results for epiphyte assemblages at each intertidal level.

| Pair | ANOSIM pairwise test | | | SIMPER result, taxa most responsible to dissimilarity |
	Global R	Significance level (%)	Dissimilarity (%)	
Upper vs middle	0.617	0.1	75.81	*Navicula* sp. (12.34%), *Cocconeis* sp. (11.85%), *M. strangulans* (10.42%), *Ectocarpus* sp. (9.62%), *Chamaecalyx* sp. (7.90%), *Nitzschia* spp. (6.55%), *Merismopedia* sp. (6.36%), *Lyngbya* sp. (5.77%), *R. arcuatum* (3.47%), *O. pulchra* (3.45%).
Upper vs lower	0.659	0.1	76.61	*Cocconeis* sp. (12.61%), *M. strangulans* (9.39%), *Ectocarpus* sp. (8.68%), *Merismopedia* sp. (8.04%), *Chamaecalyx* sp. (7.86%), *Navicula* sp. (6.82%), *R. arcuatum* (6.53%), *Nitzschia* spp. (6.06%), *Lyngbya* sp. (5.41%), *S. alsidii* (3.96%), *Ulvella* sp. (3.89%).
Middle vs lower	0.418	0.1	52.30	*Navicula* sp. (15.06%), *Cocconeis* sp. (12.29%), *M. strangulans* (10.92%), *Merismopedia* sp. (10.35%), *Chamaecalyx* sp. (9.23%).

Epiphyte distribution across host thallus sectors

A nonregular epiphyte spatial distribution was observed. The main differences were observed between the periphery–middle and the holdfast sectors (ANOSIM global $R = 0.184$, $P = 0.003$), the epiphyte abundance in the periphery and middle sectors being considerably lower than on the holdfast (Fig. 6).

All classes of epiphytes except the dinoflagellates had different abundances when comparing host sectors (Cyanophyceae ANOSIM global $R = 0.179$, $P = 0.001$; Bacillariophyceae ANOSIM global $R = 0.064$, $P = 0.001$; Phaeophyceae ANOSIM global $R = 0.059$, $P = 0.004$; Dinophyceae ANOSIM global $R = -0.014$, $P = 0.926$; Rhodophyceae ANOSIM global $R = 0.104$, $P = 0.001$; Chlorophyceae ANOSIM global $R = 0.065$, $P = 0.002$). For red algae, green algae, diatoms and cyanobacteria, the total abundances in holdfast regions were considerably higher. However, the brown algal epiphytes were more abundant in holdfast host sectors than at the periphery and middle portions (ANOSIM global $R = 0.131$, $P = 0.001$) (Fig. 6).

A differential distribution along thalli of *Ulva* sp. was observed in the following abundant species: *Lyngbya* sp., *Cocconeis* sp., *R. arcuatum*, *S. alsidii*, *O. pulchra*, *Chamaecalyx* sp. and *Merismopedia* sp. (ANOSIM global $R = 0.135$, $P = 0.003$; global $R = 0.071$, $P = 0.045$; global $R = 0.083$, $P = 0.038$; global $R = 0.114$, $P = 0.008$; global $R = 0.244$, $P = 0.001$; global $R = 0.489$, $P = 0.001$; and global $R = 0.243$, $P = 0.002$, respectively). All these epiphyte taxa were most abundant in the holdfast sectors (Fig. 7). *Chamaecalyx* sp. and *O. pulchra* (Cyanophyceae) were both absent at the host thallus periphery (Fig. 7).

The abundance of *Cocconeis* sp., *Merismopedia* sp. and *Chamaecalyx* sp. in holdfast sectors represented more than 50 percent of the total epiphyte abundance, but the abundance of *S. alsidii*, *Lyngbya* sp., *O. pulchra* and *R. arcuatum* ranged between 10% and 34% of the total abundance in this sector (Fig. 7).

SIMPER analysis made it possible to identify characteristic epiphyte assemblages at each host region. The middle region differed from the periphery by the greater abundance and/or the presence of an assemblage of *Cocconeis* sp., *Ectocarpus* sp., *Navicula* sp., *M. strangulans*, *Lyngbya* sp., *Merismopedia* sp., *Nitzschia* sp., *O. pulchra* and *R. arcuatum* (Table 3). By comparing both the periphery and the holdfast regions, *Chamaecalyx* sp., *M. strangulans*, *Navicula* sp., *Merismopedia*

sp., *Cocconeis* sp., *Lyngbya* sp. and *R. arcuatum* were the taxa most responsible for dissimilarity. The lowest dissimilarity percentage was between the middle and the holdfast regions. This dissimilarity was given by seven epiphyte taxa: *Chamaecalyx* sp., *M. strangulans*, *Navicula* sp., *Cocconeis* sp., *Merismopedia* sp., *Ectocarpus* sp. and *Lyngbya* sp. Comparing the assemblages, it was possible to detect no coincident species
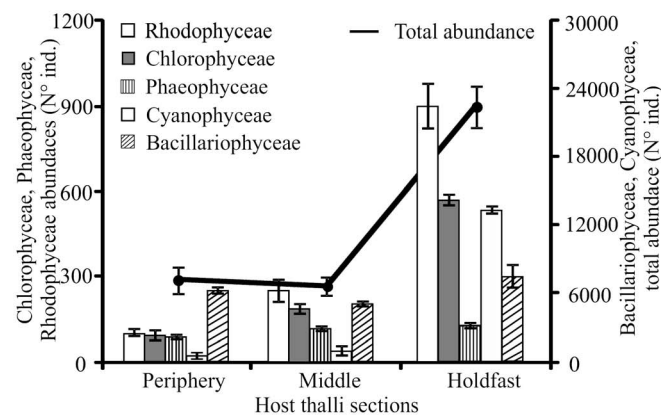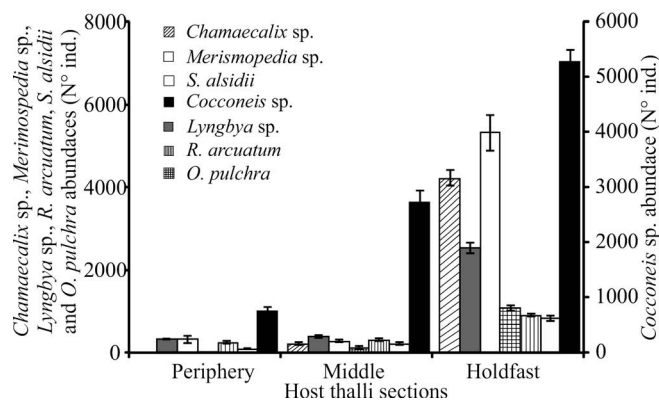

Fig. 6. Abundance across host thalli sectors. Epiphyte abundance and total abundance in the periphery, middle and holdfast *Ulva* sp. regions expressed as number of individuals.

Fig. 7. Abundance of the more abundant epiphyte taxa on different sectors of host thalli; abundance expressed as numbers of individuals.

Table 3. ANOSIM pairwise tests and similarity of percentage (SIMPER) results for epiphyte assemblages at each host region.

| | ANOSIM pairwise test | | | |
| | Global R | Significance level (%) | Dissimilarity (%) | |
Pair				SIMPER result, taxa most responsible to dissimilarity
Periphery vs middle	0.082	8.3	68.07	*Cocconeis* sp. (14.38%), *Ectocarpus* sp. (9.36%), *Navicula* sp. (8.69%), *M. strangulans* (8.29%), *Lyngbya* sp. (7.45%), *Merismopedia* sp. (7.29%), *Nitzschia* sp. (6.95%), *O. pulchra* (5.49%), *R. arcuatum* (4.56%).
Periphery vs holdfast	0.3	0.1	69.12	*Chamaecalyx* sp. (16.39%), *M. strangulans* (14.42%), *Navicula* sp. (13.38%), *Merismopedia* sp. (9.11%), *Cocconeis* sp. (7.70%), *Lyngbya* sp. (5.42%), *R. arcuatum* (4.04%).
Middle vs holdfast	0.171	07	63.33	*Chamaecalyx* sp. (16.47%), *M. strangulans* (11.23%), *Navicula* sp. (11.00%), *Cocconeis* sp. (10.15%), *Merismopedia* sp. (9.00%), *Ectocarpus* sp. (6.12%), *Lyngbya* sp. (6.08%).

between host regions, *O. pulchra* being a typical species at the middle host region (Table 3).

Species richness and species diversity

Both specific epiphyte richness and diversity varied between the intertidal elevations (ANOSIM global $R=0.09$, $P=0.018$, and ANOSIM global $R = 0.07$, $P = 0.042$, respectively). Species richness was highest at the upper levels, reaching up to 14 epiphyte taxa (ANOSIM global $R=0.193$, $P=0.006$), with respect to the middle and lower elevations. Species diversity was also higher at the upper levels (ANOSIM global $R = 0.139$, $P = 0.013$) with respect to the two other elevations. On the other hand, seasonality in species richness and species diversity was not observed (ANOSIM global $R=-0.037$, $P = 0.854$, and ANOSIM global $R = -0.032$, $P = 0.800$, respectively). A zone gradient was only observed in species richness (ANOSIM global $R = 0.151$, $P = 0.001$). The host holdfast zone had a higher number of epiphyte taxa (ANOSIM global $R = 0.264$, $P = 0.001$) compared to the middle and periphery regions.

Relationships of host attributes with epiphytic load in relation to environmental factors

In the PCA analysis, the first two axes explained 83.8% of the joint variation of environmental factors and biological variables. PC 1 was related mainly to the seasonal variability of the samples. However, the samples grouped with respect to the intertidal elevations were not differentiated by either PC axes. Samples from the warmer months (November–March) were grouped on the more negative side of PCA axis I; whereas, samples from the cold period (May–October) were distinguished on the more positive side of PCA axis I. In addition, the right side of PC 1 was characterized by high epiphyte abundance, frequent periods with high seawater temperatures, long days and high radiation. With respect to the host, *Ulva* sp., thalli were characterised by high values in both the maximum diameter and the wet weight of fronds. Cover of *M. strangulans* was not correlated with either cold or warm periods, indicating that this epiphyte species did not show seasonality (0.8).
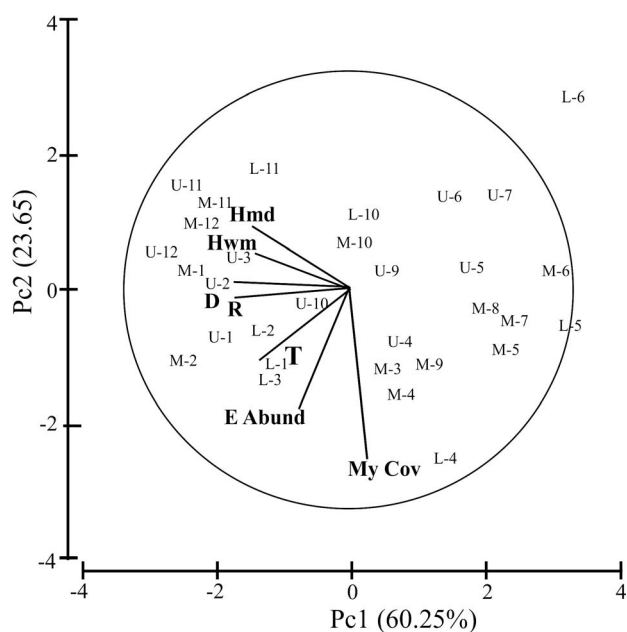
M. strangulans on *Ulva* sp

Myrionema strangulans thalli were observed as brown spots on epidermic cells, extended on different sectors of the host fronds (Fig. 9). Immature *M. strangulans* individuals presented a monostromatic basal disk made up of filaments radiating from the central area to the periphery (Fig. 10). Mature thalli formed erect uniseriate filaments on different epiphyte sectors (Figs 11, 12).

M. strangulans along gradients

Cover of *M. strangulans* varied when comparing the three intertidal levels (ANOSIM global $R=0.001$, $P=0.429$). Thalli of *M. strangulans* were found at the upper, middle and lower elevations. Furthermore, no differences were observed in cover of *M. strangulans* between seasons (ANOSIM global R
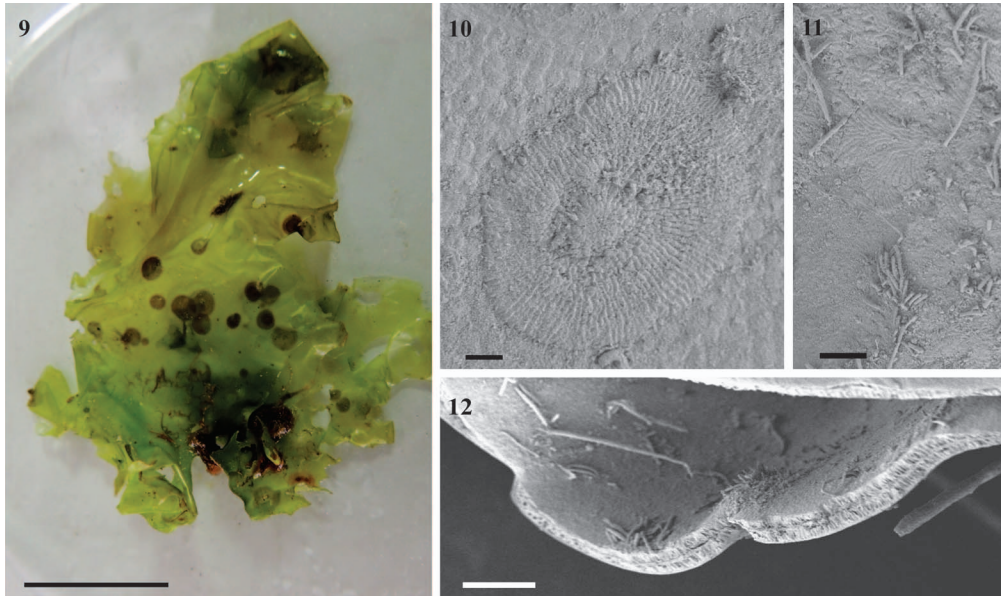

Fig. 8. PCA biplot: association between biological and environmental variables. Biological variables: maximum diameter host (Hmd), wet mass host thalli (Hwm), epiphyte abundance (E Abund) and *Myrionema strangulans* cover (My Cov). Environmental variables: seawater temperature (T), radiation (R) and day length (D). Months sampled were indicated by numbers: January (1), February (2), March (3), April (4), May (5), June (6), July (7), August (8), September (9), October (10), November (11) and December (12). Intertidal elevations were indicated by letters: upper (U), middle (M) and lower (L).

Fig. 9. *Myrionema strangulans* on thallus of *Ulva* sp.. *M. strangulans* appears as dark spots spread out on the host surface. Scale bar = 3 cm.
Fig. 10. SEM photomicrograph of immature *M. strangulans* showing the monostromatic basal disk, constituted by radiating filaments. Scale bar = 20 μm.
Fig. 11. SEM photomicrograph of mature *M. strangulans* thalli formed by erect uniseriate filaments on the host surface. Scale bar = 100 μm.
Fig. 12. SEM photomicrographs of a transverse section of a distromatic *Ulva* thallus. *M. strangulans* filaments can be seen on the surface. Scale bar = 100 μm.

= 0.015, P = 0.135). However, an uneven distribution was observed comparing host thalli (ANOSIM global R = 0.14, P = 0.001) since higher cover of *M. strangulans* was reported on holdfast sectors.

DISCUSSION

By comparing the list of epiphyte taxa in this study and those of other hosts from the Patagonian coast, we see that most epiphytes have already been mentioned as generalist epiphytes (Gauna *et al.* 2014). These results suggest that the algal epiphyte flora on macroalgae is determined mostly by the geographical area in which they grow and not by the host. The algal epiphyte community on *Ulva* sp. on the Patagonian coast consisted of a considerable number of species. In the green host *Codium vermilara* (Olivi) Delle Chiaje from the North Patagonian coast, a comparable number of epiphytes were recorded (Miravalles 2008). The epiphyte assemblage composition as well as the patterns of distribution of the most abundant species showed considerable variation in space and time. Only a few taxa were distributed uniformly, spatially and temporally. The analysis performed here indicated that in order to understand the sources of variation of both epiphyte distribution and abundance, the necessary emphasis was on spatial and temporal scales and host morphology. In addition, this study revealed the importance of gradients in explaining the host spatial variation and those of the epiphyte communities.

The results indicate that the epiphyte community on thalli of *Ulva* sp. was characterised by an assemblage of a few stable algal species and that the high epiphyte diversity was due mainly to occasional species. Among the taxa with the highest incidence rates, found six frequent species and also a total of eight very abundant species during the course of this study, and of these, five were recorded with a high incidence rate. Diatoms had the highest number of epiphyte taxa. This was expected since diatoms, together with bacteria, are normally marine primary colonisers that play a crucial role in subsequent attachments and consequently in the final structure of algal communities (Kumar *et al.* 2011). The most represented taxa were the epiphytic genera *Navicula* and *Nitzschia*. Since they are not firmly attached to the substratum and are likely to be suspended by currents, these genera include highly mobile species with greater colonisation ability (Hudon & Legendre 1987).

The maximum epiphyte abundance was recorded at the mid-intertidal elevation; although, the highest species richness and diversity were reported from the upper elevations. Interestingly, we found the highest abundances in the holdfast host zone, a position that should provide favourable shade conditions for epiphyte development. On the contrary, Arrontes (1990) reported that *Fucus vesiculosus* Linnaeus at middle elevations presented a lower number and abundance of epiphytes. This observation was also reported by D'Antonio (1985) in *Rhodomela larix* (Turner) C.Agardh. Both Arrontes (1990) and D'Antonio (1985) explained the low abundance as due to environmental stress based on high insolation during the low-tide period.

Differences between assemblages at different elevations were also attributable to a gradient of wave exposure since upper elevations suffer less from the effect of waves (Raffaelli & Hawkins 1996; Garbary 2007). Wave exposure has also been considered a major factor influencing the structure of algal assemblages, and scales of exposure have been based on the composition of benthic communities

(Ballantine 1961). *Ulva* sp. on the Patagonian coast are particularly known for showing preference for sheltered elevations (Rico *et al*. 2005).

Considering individually the epiphyte species in this study, *R. arcuatum* is the only one for which a consistent effect of elevation was found and, consequently, for which a consistent effect of wave exposure can be hypothesised. This species was present at the middle and lower elevations, indicating that it could be a species adapted to medium to high wave exposure.

No seasonality of epiphyte abundance was detected for species richness or species diversity. These results indicated that growing *Ulva* thalli may be colonised by different epiphytes in any season at an equal intensity. This condition was accompanied by the clear biomass seasonality observed in *Ulva* growing at upper intertidal levels, which were both larger and heavier, in parallel with the seasonality of the epiphyte abundance detected in different epiphyte taxa.

An unequal distribution of epiphytes was also reported on the green macroalgae hosts, *Codium isthmocladum* Vickers and *Anadyomene stellata* (Wulfen) C.Agardh (Ballantine 1979). This particular distribution was attributed to many factors: light and water motion in different host regions, including shading effects; uneven distribution of compounds which might act as epiphyte deterrents; different ages and therefore different amounts of time available for an epiphyte community to develop; and differences in surface tension and roughness of the host thallus (Longtin *et al*. 2009). In macroalgae with apical meristematic zones, epiphytes usually show clear zonation patterns, and the number of species usually decreases towards the younger parts (Ballantine 1979). This is not the case in *Ulva* since they show diffuse growth, so it is impossible to infer tissue ages; nonetheless, it has been possible to observe rapid-growth regions (newer tissues) and slower-growth regions (older tissues) (Krumhansl *et al*. 2015).

Among all the epiphytes observed in this study, *M. strangulans* was the most evident to the naked eye. Its cover did not present any variation between the three intertidal levels since thalli were found growing in upper, middle and lower elevations. Furthermore, although no differences were observed between seasons, an uneven distribution was observed when comparing host thallus sectors since a higher covering of *M. strangulans* was clear on the holdfast region. Kornmann & Sahling (1983) established that *M. strangulans* grows widely in temperate seas and can produce profuse growth on *Ulva* elsewhere. This species has also been reported as an important epiphyte in *Ulva* farms in South Africa (Bolton *et al*. 2009). *Myrionema* outbreaks have been reported under farm conditions during spring and summer. The epiphyte causes severe infection on *Ulva*, eventually causing the *Ulva* thalli to disintegrate (Bolton *et al*. 2009). Competition between hosts and their seaweed epiphytes has been demonstrated under natural and artificial growth conditions (Arrontes 1990; Friedlander & Ben-Amotz 1991; Svirski *et al*. 1993), and the extent of the damage was determined by the intensity of the infections (Cancino *et al*. 1987; Buschmann & Gomez 1993; Siniscalchi *et al*. 2012). In nature, *M. strangulans* did not show any variation across seasons. It is probable that the incidence and abundance of

M. strangulans are different from farm conditions, possibly associated with a nutrient (Bolton *et al*. 2009)

To conclude, on Northern Patagonian coasts of Argentina, the studied factors – intertidal elevations, seasons and frond host regions – contributed significantly to the variability in the species composition and dominant structure of epiphytes on *Ulva* sp. Our findings indicate that these factors play a key role in explaining the epiphyte and host distribution in intertidal systems. Additional studies incorporating populations of *Ulva* sp. from other areas are necessary to verify whether the patterns observed here can be extrapolated for the whole Patagonian coast.

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