



Short communication

The establishment of the non-native seaweed *Polysiphonia morrowii* in Northern Patagonia: Size of thallus and reproduction

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ABSTRACT

A population of *Polysiphonia morrowii*, recently introduced in the South West Atlantic Ocean, was studied to determine the degree of its establishment. Monthly variations of: thallus size, proportion of life history phases, and tetrasporangia production, were evaluated from June to October 2010. The erect thalli appeared in autumn, and decayed in spring when they became overgrown by epiphytic diatoms. Vegetative thalli were usually smaller than fertile thalli. In spring, the thalli had the maximum mean values of length (199.5 mm), thickness (52 mm) and dried weight (4 g). Although tetrasporophytes represented between 73% and 90% of the thalli collected each month, a few cystocarpic thalli were also found. Variations of thallus size and proportion of life history phases were also studied between 2010 and 2014. The inter-annual increase in the proportion of the tetrasporophytic phase and the progressive increase of the size of *P. morrowii* thalli indicated an ongoing establishment of the population in this habitat.

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1. Introduction

The genus *Polysiphonia* comprises species of filamentous macroalgae with a widespread distribution and a variety of life habits (Fralick and Mathieson, 1975; Guiry and Guiry, 2016). The dispersal and acclimation capacity together with the morphological plasticity of some species of *Polysiphonia* has led to their introduction in several coasts where they are considered as invasive (Eno, 1996; Mclvor et al., 2001; Sliwa et al., 2009).

Polysiphonia morrowii is a conspicuous species because the thalli can attain a great size (Kudo and Masuda, 1992; Curiel et al., 2002), and has been listed as one of the 'worst' marine invasive species in the Mediterranean (Streftaris and Zenetos, 2006), presumably dispersed through mariculture activities. It is native from the coasts of Japan, China and Korea (Yoon, 1986; Kudo and Masuda, 1992), but it has been found in the Atlantic Ocean, the Mediterranean Sea and the South Pacific Ocean (Curiel et al., 2002; Kim et al., 2004; Geoffroy et al., 2012; Mamoozadeh and Freshwater, 2012; Croce and Parodi, 2014; Raffo et al., 2014).

P. morrowii was discovered in recent years at the coastal marshes of northern Patagonia (Argentina), forming extensive patches of 340 g m⁻² (wet weight) and living in epizootic association with the Japanese oyster *Crassostrea gigas*, a highly invasive species which is known to be colonizing the Patagonian coasts (dos Santos and Fiori, 2010). Considering that *P. morrowii* is the second introduced marine species that has been recorded in this habitat, the monitoring of this population must be addressed.

This study was conducted to investigate the periodicity of *P. morrowii* in northern Patagonia, with the aim to determine the degree of establishment of this population. The monthly and inter-annual variability of length, thickness and dried weight of thalli were evaluated as well as the reproductive periodicity of the population.

2. Materials and methods

The collection of thalli was carried out in a shallow coastal marsh located in Los Pocitos, northern Patagonia (40° 26' 14" S; 62° 25' 8" O). The marsh is formed by seagrass meadows and reefs of the Pacific oyster *Crassostrea gigas* (Borges, 2006).

Preliminary monthly observations were carried out to determine the collection period. *P. morrowii* lived in the intertidal pools that arise in the oyster reefs, always covered with water, being more

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Table 1
Water surface temperature, daylength and concentration of silicate in the water obtained at noon, and mean values of vegetative and reproductive variables of *Polysiphonia morrowii* from June to October 2010 in northern Patagonia. nd = not detected; (\pm) = standard deviations.

Month	Water temperature ($^{\circ}$ C)	Daylength	Silicate (μ mol L $^{-1}$)	% Fertile thalli	No. fertile branchlets	No. tetrasporangia per fertile branchlet
Jun	8	9 h 16 m	16.23	73.3	2.96 (\pm 2.6)	3.28 (\pm 1.5)
Jul	9.2	9 h 33 m	19.46	73.3	1.81 (\pm 1.4)	3.09 (\pm 1.7)
Aug	11	10 h 33 m	25.08	75	1.63 (\pm 1.2)	3.19 (\pm 1.4)
Sep	12	11 h 45 m	10.63	90	1.74 (\pm 1.6)	3.19 (\pm 1.5)
Oct	20.5	13 h 1 m	13.78	76.5	0.95 (\pm 0.7)	2.72 (\pm 1.2)

abundant in the lower intertidal. The erect thalli were found from autumn to spring.

Thirty attached thalli were haphazardly collected at monthly intervals from June to October 2010. Additionally, 20 thalli were collected in September of 2011, 2013 and 2014.

Water surface temperature was measured at noon, and daylength was estimated from time records of sunrises and sunsets. Water samples were collected at noon at a depth of 0.5 m to determine silicate concentration in the water.

Each thallus was rinsed with filtered seawater and examined under a Nikon Eclipse 80i microscope to determine life history stage.

The size of each thallus was determined through the following variables: dried weight (DW), after drying in an oven at 60 $^{\circ}$ C for 48 h up to constant weight; length, measured from the basal prostrate axes to the end of the longest erect axis of the thallus; and thickness, measured with a caliber at the bulkiest part of each thallus.

For each tetrasporophytic thallus collected in 2010, the monthly production of tetrasporangia was determined. A total of 10 fragments of 5 mm were sectioned from the apical part of each fertile thallus. In each fragment, the number of fertile branchlets was counted. The number of tetrasporangia per fertile branchlet was estimated based on 75 fertile branchlets from all fragments sectioned per month.

Water samples were vacuum filtered through Whatman GF/C glass fiber and the concentration of silicate was determined with an autoanalyzer Technicon II.

Differences in length, thickness, and DW of *P. morrowii* thalli between sampling times were analyzed through ANOVA and Tuckey poshoc comparisons, after testing for normality and homoscedasticity, without the need of transformations.

The percentage of fertile and vegetative thalli of the population was calculated for each month and year, and the monthly and inter-annual variation of this percentage was evaluated through goodness of fit with χ^2 . The monthly variation of the production of tetrasporangia was evaluated in the samples collected in 2010 through generalized linear models (GLM) with quasipoisson errors, Tuckey comparisons and frequency of Poisson distribution.

Statistical analyses were done with R software (R Development Core Team, 2011).

3. Results

Thalli collected from June to September were dark red and slender with few epiphytic diatoms colonies, while those collected in October were clustered, brownish, and were almost completely covered by epiphytic diatoms.

Silicate levels ranged between 10 μ mol L $^{-1}$ and 25 μ mol L $^{-1}$, with a maximum in August (Table 1).

The vegetative thalli were larger in September and October ($F=6.02$; $df=4$; $p=0.002$), whereas the fertile thalli showed no variation in length among months ($F=1.97$; $df=4$; $p=0.11$) (Fig. 1a). Fertile thalli were larger than vegetative thalli in June ($F=8.61$; $df=1$; $p \ll 0.01$) and August ($F=4.35$; $df=1$; $p < 0.05$).

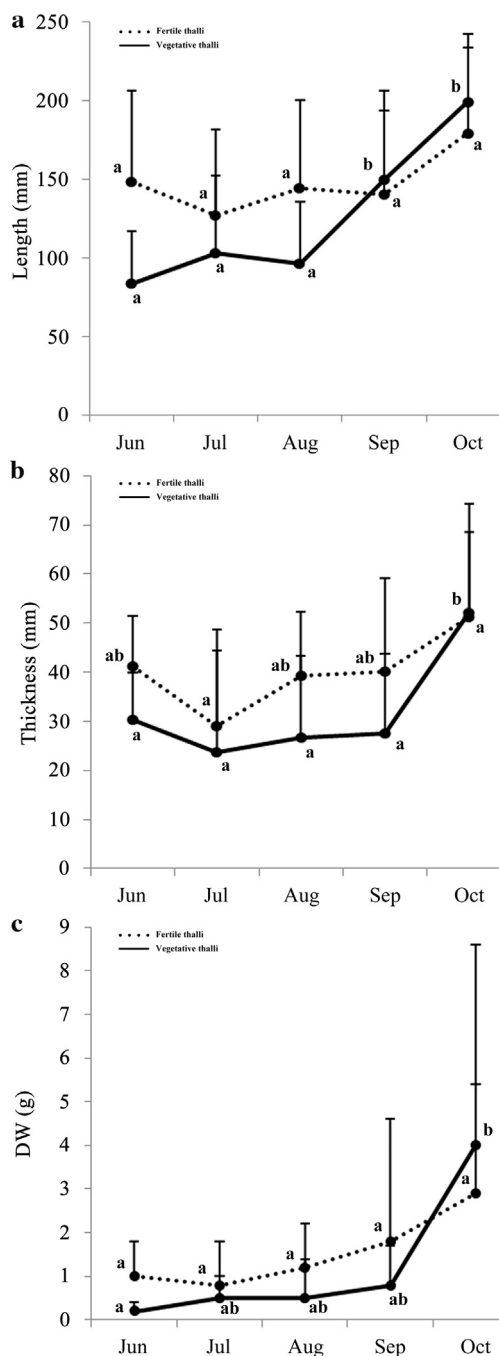


Fig. 1. Mean monthly variations of size of *Polysiphonia morrowii* thalli during 2010: (a) length; (b) thickness; (c) dried weight (DW). Vertical bars represent standard deviations (\pm SD). Letters above and below each line represent Tuckey poshoc comparisons among months. $n=30$.

The fertile thalli were thinner in July and thicker in October ($F=4.17$; $df=4$; $p=0.004$) (Fig. 1b). On the contrary, the vegetative thalli showed no variation in thickness among months ($F=1.79$; $df=4$; $p=0.17$). The thickness of fertile thalli was higher than that of vegetative thalli in June ($F=6.67$; $df=1$; $p<0.05$) and August ($F=4.39$; $df=1$; $p<0.05$).

The DW of vegetative thalli was lowest in June and highest in October ($F=2.84$; $df=4$; $p=0.05$); however there were no significant differences in this variable for fertile thalli ($F=1.58$; $df=4$; $p=0.19$) (Fig. 1c). In June, DW of fertile thalli was higher than that of vegetative thalli ($F=5.17$; $df=1$; $p<0.05$).

From all thalli collected, 77% were fertile: 76% with tetrasporangia and 1% female bearing cystocarps. No significant differences were found in the proportion of fertile and vegetative thalli among months ($0.12 < p < 1$) (Table 1).

The number of fertile branchlets per apical fragment was highest in June and lowest in October ($p<0.001$) (Table 1). The majority of fertile thalli had between 1 and 5 fertile branchlets per apical section (90% of the thalli examined). In June, July and August the majority of apical sections had 2 or 3 fertile branchlets, while in September and October, it was more frequent to find only one fertile branchlet per apical fragment. The number of tetrasporangia per fertile branchlets varied between 1 and 13. The mean number of tetrasporangia was lowest in October ($\chi^2 = 6650.2$; $df=4$; $p \ll 0.01$) (Table 1). The 94% of the fertile branchlets had between 1 and 5 tetrasporangia.

The length and thickness of *P. morrowii* thalli significantly increased along the years. The length was lowest in 2010 and reached a maximum of 210 mm in 2013 ($F=5.6$; $df=3$; $p=0.001$); while the thickness reached a maximum of 59 mm in 2014 ($F=14.9$; $df=3$; $p \ll 0.01$). Dried weight was not significantly different among years ($F=0.35$; $df=3$; $p=0.80$).

The proportion of fertile thalli was similar among years ($\chi^2 = 1.35$; $p=0.72$): 90% in 2010 (85% tetrasporophytes and 5% cystocarpic), 85% in 2011 (all tetrasporophytes), 95% in 2013 (75% tetrasporophytes and 15% cystocarpic), and 100% in 2014 (all tetrasporophytes).

4. Discussion

This study provides the first results about the temporal variation of thallus size and reproduction of *Polysiphonia morrowii* in the South Atlantic Ocean.

The population of *P. morrowii* had a marked annual cycle of growth in northern Patagonia similar to that reported for the non-native populations located in the Mediterranean Sea and in France (Curiel et al., 2002; Geoffroy et al., 2012). *P. morrowii* grew and reproduced between winter and spring (June to October), denoting its affinity with cold-temperate waters (Kudo and Masuda, 1992) but the erect thalli did not remain until summer as in its native range (Kudo and Masuda, 1981). The rise of water temperature in middle spring would have driven the population from northern Patagonia to have a shorter annual cycle compared to the native range. The strategy for establishment of this species in northern Patagonia may be attained by a rapid growth during the colder months and by the permanence of quiescent creeping axes on oyster's shells during the summer.

Culture and field studies have found a positive relationship between temperature and the increase of thallus size of *Polysiphonia* species (Edwards, 1970; Subba Rangaiah et al., 2003). In this study, the size of the thalli remained constant during the colder months, and reached maximum values in spring. Rather than a positive influence of temperature on the vegetative growth of the thalli, the larger thalli found in spring could be the result of low rate of recruits or a longer period to grow.

In its native range, *P. morrowii* grows in cool-temperate waters between winter and summer (December to August), achieving maximum size and reproduction in late winter-early spring (Kudo and Masuda, 1981; Yoon, 1986; Kim et al., 1994). The thalli of *P. morrowii* from northern Patagonia were larger than Japanese and Korean specimens (Kudo and Masuda, 1981; Kim et al., 1994).

Changes in daylength promote the development and reproduction of red and brown algae (Edwards, 1970; Dring, 1988). In our study, the interaction between daylength extension and temperature increase was related to the maturation of tetrasporophytes of *P. morrowii*, similarly to its native range, where the maturation of tetrasporangia increases towards the summer (Kudo and Masuda, 1981).

The high epiphyte loads on thalli collected in October contributed to their higher DW and thickness. The levels of silicates in the water were drawn down in September and October, evidencing the proliferation of diatom colonies over the thalli. Epibiosis by diatoms is a significant factor in the study site during the spring (Borges, 2006) that must be considered in further studies. These results are in agreement with the epiphytism reported by Curiel et al. (2002) for the Mediterranean population of *P. morrowii* in the same season.

The limiting effect of temperature on the population of *P. morrowii* could be either direct, through cellular damage, or indirect by promoting epiphytism which interferes with the physiological capabilities of *P. morrowii*.

As expected, a relationship between fertility and size of thallus was evident given that fertile thalli were usually longer, thicker and weighted more than vegetative thalli. Difference in length of the thalli between life history phases was also denoted for Korean specimens of *P. morrowii* (Kim et al., 1994).

Only a small proportion of the population of *P. morrowii* accomplished a complete triphasic cycle. Vegetative growth from prostrate axes was probably more rapidly accomplished than growth from individual propagules. This reproductive strategy is frequent in *Polysiphonia* spp. and in *P. morrowii* in particular (Davis and Wilce, 1987; Kim et al., 2004; Geoffroy et al., 2012).

Although several studies have registered all life history phases of *P. morrowii* (e.g. Yoon, 1986; Kim et al., 1994; Curiel et al., 2002), spermatangia were not found in the collected material, and fertilized female gametophytes were scarce. Sexual cycles are rare in the natural populations of *Polysiphonia* (Yarish and Edwards, 1982), and the absence of male specimens has frequently been reported (Schneider and Searles, 1991; Rindi et al., 1999; López et al., 2004; Stuercke and Freshwater, 2008). The period of occurrence of fertile males of *P. morrowii* is very short in its native range, where only a few reproductive males appear in one month (Kim et al., 1994). The absence of fertile male gametophytes in the collected material was either related to an exceptional low frequency of occurrence of this phase in the period studied, in agreement with the low frequency of cystocarpic thalli, or to a rapid defoliation of spermatangia after the transportation of thalli.

The tetrasporic phase was clearly dominant throughout the period studied, a tendency frequently reported in the Ceramiales (Thorner, 2006). The majority of phenological studies about *P. morrowii* mention a high occurrence of asexual life stages (Kim et al., 1994; Curiel et al., 2002; Geoffroy et al., 2012), that has been attributed in part to reproduction strategies (Engel et al., 2001). Asexual life cycles are usually the strongest strategy of invasive macroalgae species (Lodge, 1993; Chapman, 1999). The high abundance of tetrasporic thalli was likely to be the result of re-growth from creeping prostrate axes of older tetrasporic thalli, rather than of carposporic origin. Assuming a relatively recent introduction of this species in Argentina, it is expected that the sexual phases of the triphasic life cycle are not well developed in this habitat.

The increase in thallus length and thickness along the years and the evidence of sexual reproduction suggest an ongoing establishment of *P. morrowii* in the oyster reefs of northern Patagonia.

Polysiphonia morrowii co-occurred in this habitat with native species of *Punctaria*, *Scytosiphon*, *Ectocarpus*, *Ceramium*, *Gelidium*, *Cladophora*, *Ulva* and *Bryopsis*. The interaction between *P. morrowii* and the native seaweed community should be addressed in further studies.

The potential of invasiveness of *P. morrowii* has been demonstrated in other coasts of the world, therefore it is important to monitor this north Patagonian population in the long term and also to track the origin of its introduction.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.aquabot.2016.09.002>.

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