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Phenology of an agarophyte *Gracilaria birdiae* Plastino and E.C. Oliveira (Gracilariales, Rhodophyta) in Northeastern Brazil

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Article

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Abstract: The reproductive phenology and thallus length of *Gracilaria birdiae* were studied over a period of 12 months in a natural bed in Northeastern Brazil. Fertile specimens of *G. birdiae* were observed during the entire study period. Tetrasporophytes were the most common with an annual mean of 80.1±5.6%, followed by cystocarpic plants (9.3±3.4%), male gametophytes (8.3±3.6%) and infertile plants (2.2±3.4%). Only male gametophytes and infertile plants showed a variation in occurrence frequency during the year ($p<0.05$). With respect to thallus length, a distinct seasonal variation was observed for all reproductive stages ($p<0.05$), with the highest values recorded during the rainy season (March to August) and the lowest in the dry season (September to February). The results demonstrate that the size of individuals in this population is significantly affected by the periodic changes in the environment caused by rainfall regimes and hydrodynamism.

Introduction

The genus *Gracilaria* (Gracilariales, Rhodophyta) is widely distributed in tropical and temperate regions worldwide and is represented by more than 110 species (Critchley, 1993; Oliveira & Plastino, 1994). This genus has significant economic importance in several countries due to its agar content and rapid growth (Armisen, 1995), currently accounting for around 65% of world agar production (McHugh, 2002). *Gracilaria* has a "*Polysiphonia*-type" life history, with morphological similarities between gametophytic (n) and tetrasporophytic (2n) phases. However, the occurrence of different reproductive structures in a same thallus can be observed, which may be related to mechanical (*in situ* tetraspore germination), genetic or cytological failures (Oliveira & Plastino, 1994; Kain (Jones) & Destombe, 1995).

Studies conducted on natural populations of *Gracilaria* demonstrate a greater predominance of tetrasporophytic forms, in addition to indicating a lower amount of male than female gametophytes (Marinho-Soriano et al., 1998; Orduña-Rojas & Robledo, 2002; Polifrone et al., 2006). This variation is related mainly to different survival and fertility rates between haploid and diploid individuals (Richerd et al., 1993). The occurrence frequency of *Gracilaria* reproductive stages has been determined for several species in geographically distinct regions. In the tropics, fertile individuals are present in populations throughout the year, while in temperate regions fertility peaks are restricted to one or two periods, generally when temperatures and irradiances are highest (Kain (Jones) & Destombe, 1995; Espinoza-Avalos, 2005). Thus, some species of *Gracilaria* exhibit reproductive strategies that allow synchrony between fertile periods and favorable environmental conditions for their perennity (Marinho-Soriano et al., 1998). In Brazil, phenological studies on the genus *Gracilaria* are relatively scarce (Pinheiro-Joventino & Bezerra, 1980; Plastino, 1985; Silva et al., 1987; Araújo, 2005).

Gracilaria birdiae Plastino and E.C. Oliveira, one of the main sources of agar in the country, is found from the coast of Ceará State (3ºS) to Espirito Santo State (20ºS), Brazil, in relatively abundant beds of perennial seaweeds (Plastino & Oliveira, 2002). This species has recently been the focus of several laboratory studies (Plastino, 2004) and its life history has been completed in a laboratory culture (Costa & Plastino, 2001). Strain selection and the physiological demands of the strains were performed with the aim of improving cultivation techniques in the sea (Costa & Plastino, 2011; Ursi & Plastino, 2001; Ursi et al., 2003; Plastino et al., 2004; Ursi et al., 2008). Studies related to the cultivation of this species have been conducted in different environments such as shrimp ponds (Marinho-Soriano et al., 2009; Oliveira, 2007), estuaries (Marinho-Soriano et al., 2006) and the sea (Bezerra & Marinho-Soriano, 2010), showing promising growth rates.

In the state of Rio Grande do Norte, extractivist harvesting of *G. birdiae* is performed by coastal communities, playing an important role in the local economy. Harvest occurs throughout the year during low tides and the short time interval between one harvest and the next is not sufficient to permit the complete recovery of seaweed beds. Thus, this activity is mainly responsible for the gradual decrease of seaweed stocks in the region, making the situation of these natural resources in the country increasingly worrisome. The objective of the present study was to estimate the reproduction of a natural population of *G. birdiae* based on the occurrence of different reproductive stages over a period of one year, as well as variations in thallus length as a function of seasonal environmental changes.

Materials and Methods

Site location and collection

A perennial infralittoral population of *Gracilaria birdiae* was studied at Rio do Fogo Beach (5°15'41"S-35°23'11"W), Rio Grande do Norte State, located on the Northeast coast of Brazil (Figure 1), between January and December, 2008, in an area measuring approximately 600 m^2. The Rio do Fogo River discharges near the *G. birdiae* bank (at a distance of approximately 500 m) and, depending on the rainfall regime, accounts for the salinity and nutrient fluctuations recorded there. Two distinct seasons were identified at this site: (i) rainy, from March to August, and (ii) dry, in January and February and between September and December.

Samples of *G. birdiae* were obtained along a 10 m transect, from which twenty points were randomly selected. The collection procedure was repeated three times, totaling sixty sampling points each month. The individuals selected were measured directly in the field and samples of each specimen were collected and stored in plastic bags for laboratory analysis. Specimens were separated according to their reproductive stage (tetrasporophytes, cystocarpic plants, and male gametophytes). Tetrasporophytes and male gametophytes were identified with the help of histological cuts visualized under a microscope, while cystocarpic plants were identified with the naked eye by the presence of cystocarps on the thallus. Thalli without tetrasporangia, spermatangial conceptacles, and cystocarps were considered to be infertile.

Environmental parameters

Environmental parameters (water temperature, salinity, pH, and water transparency) were recorded monthly by using a multiparameter probe (Horiba U-10) and a Secchi disk, simultaneously with seaweed collection. Water samples (300 mL) were collected at the site, fixed (2M H_2SO_4), filtered (Whatman GF/C) and frozen until analysis. Dissolved inorganic nitrogen (DIN = NH_4^+ + NO_3^-

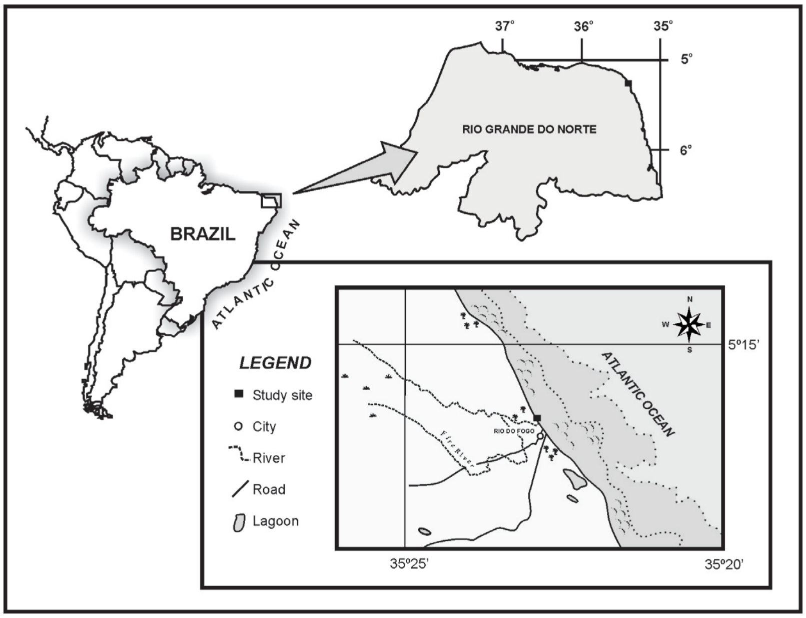

Figure 1. Map showing the study location: Rio do Fogo Beach, Rio Grande do Norte State, Brazil.

+ NO_2^-) and orthophosphate (PO_4^{3-}) were analyzed according to methods described by Strickland & Parsons (1972). Rainfall and photosynthetically active radiation (PAR) were obtained from INPE (Brazilian National Space Research Institute). Rainfall values correspond to monthly accumulation (mm/month) and monthly PAR to daily means of values obtained every 30 min.

Statistical analyses

Seasonal variations in the thallus length at different reproductive stages of *Gracilaria birdiae* and in the environmental parameters recorded during this study were analyzed using one-way ANOVA. When significant differences were detected, the post-hoc Student-Newman-Keuls test was used. Student's t-test was applied to identify the differences between the rainy (March to August) and dry (September to February) seasons. Pearson's correlation was used to determine relationships existing between thallus length and the recorded environmental parameters. Sexual proportion analysis (cystocarpic plants X male plants) was conducted using the χ^2 test. Data were analyzed for normality and homoscedasticity using the Kolmogorov-Smirnov and Levene tests, respectively. Data that did not meet the test premises were transformed (log 10, ln, and square root) or evaluated using the corresponding nonparametric tests.

Results

Environmental parameters

Environmental parameters recorded during this study generally exhibited different behavior in the two seasons (Table 1). Mean temperature values for the rainy and dry seasons were 26.6±0.5 and 28.7±0.46 ºC, respectively (t=3.08; $p<0.05$). Salinity was constant during the dry season, with a mean of 34.8±0.4 PSU, while during the rainy season there was a significant reduction from 35 to 27 PSU (F=2960; $p<0.05$). Water transparency was negatively related to wind speed (r=-0.92; $p<0.05$), reaching its highest values at the beginning of the year (maximum in May of 135 cm). A significant reduction was observed after this period, decreasing to 15 cm (September).

With regards to nutrients, DIN (NO_2^- + NO_3^- + NH_4^+) was higher during the rainy season, with the maximum value recorded in April (9.66±0.07 µM). Mean values for the rainy and dry season were 3.85±3.88 and 1.49±0.87µM, respectively (t=2.67; $p<0.05$). NH_4^+ was the nutrient that contributed most to the final DIN concentration, except for April (44.8%) and December (24.9%), when the NO_3^- concentration was higher (54.5 and 63.4%, respectively). NO_2^- contributed the least to the DIN and showed no difference between the two seasons (t=0.63; $p>0.05$). PO_4^{3-} exhibited significant variations throughout the year (F=34.40; $p<0.05$), with the minimum value recorded in January (0.08±0.013 µM) and the maximum in July (1.76±0.03 µM). This nutrient showed a negative correlation with water transparency (r=-0.82; $p<0.05$) and a positive correlation with wind speed (r=0.75; $p<0.05$).

Table 1. Environmental parameters (range and mean±SD) recorded during the rainy and dry seasons of 2008.

	Dry season		Rainy season		t-value	*p*
	Range	Mean±SD	Range	Mean±SD		
Rainfall (mm/month)	0.2-67.8	26.8±23.7	286.3-542.0	426.4±89.6	19.46	<0.001
PAR (µmol.photons. m^{-2} s^{-1})	1158.9-1436.6	1284.2±98.27	416.8-1077.2	860.0±235.8	13.01	<0.001
Wide speed (m.s^{-1})	4.74-6.00	5.32±0.43	3.14-5.01	4.07±0.80	3.37	<0.05
Water temperature (ºC)	27.0 -29.0	28.7±0.46	26.0-29.0	26.6±0.55	3.08	<0.05
Salinity (PSU)	34.0-35.0	34.8±0.4	27.0-35.0	33.3±3.1	2.14	<0.05
pH	7.6-8.7	8.2±0.0	7.5-8.3	7.8±0.3	3.15	<0.05
Water transparency (cm)	15.0-90.0	47.0±29.4	30.0-135.0	79.6 ±38.9	3.86	<0.001
NO_2 (µmol.L^{-1})	0.04-0.17	0.11±0.06	0.05-0.19	0.10±0.06	0.63	0.535
NO_3 (µmol.L^{-1})	0.01-0.64	0.23±0.30	0.01-5.26	1.17±2.05	2.05	<0.05
NH_4 (µmol.L^{-1})	0.25-2.34	1.15±0.83	0.60-6.48	2.58±2.38	2.54	<0.05
DIN (µmol.L^{-1})	0.50-3.04	1.50±0.87	0.79-9.66	3.85±3.88	2.67	<0.05
PO_4 (µmol.L^{-1})	0.08-1.37	0.64±0.55	0.17-1.76	0.71±0.73	0.32	0.753

Phenology of G. birdiae

Fertile specimens of *G. birdiae* were observed during the entire study period. Tetrasporophytes were found in larger amounts, with an annual mean of 80.1±5.6%, followed by cystocarpic plants (9.3±3.4%) and male gametophytes (8.3±3.6%). Only 2.2±3.4% of the sampled individuals were infertile (Figure 2). The frequencies recorded for tetrasporophytes and cystocarpic plants were similar throughout the year (p>0.05), while male gametophytes (max. 11.7±2.9% and min. 1.7±2.9%) and infertile plants (max. 10.0±0.9% and min. 0.0±0.0%) showed significant variations (p<0.05). The proportion between female (cystocarpic plants) and male gametophytes was 1:1 in most of the months studied. In September, the occurrence of male plants was higher than cystocarpic plants (1.8♂ : 1♀; χ^2 = 8.16; p<0.05), while in February (1♂ : 2♀; χ^2 = 11.11; p<0.05) and October (0.1♂ : 1♀; χ^2 = 66.94; p<0.05) it was the inverse, that is, cystocarpic plants were more numerous.

With respect to thallus length, a distinct seasonal variation (p<0.05) was observed in all fertile stages, with the highest values recorded during the rainy season (tetrasporophytes-41.4±1.7cm (July); cystocarpic plants, 41.8±5.0 cm (July); male gametophytes, 37.9±8.7 cm (June)) (Figure 3 and Table 2). Mean thallus lengths for the rainy and dry seasons were 38±3.1 cm and 28.1±4.1 cm, (tetrasporophytes, t=8.01, p<0.05), 35.4±4.2 cm and 28.3±5.5 cm (cystocarpic plants, t=3.59, p<0.05), and 34.6±2.9 cm and 22.1±4.6 cm (male gametophytes, t=4.52, p<0.05). Thallus lengths of the three fertile stages showed positive correlations with rainfall (tetrasporophytes, r=0.69, p<0.05; cystocarpic plants, r=0.67, p<0.05; male gametophytes, r=0.83, p<0.05) and negative correlations with PAR (tetrasporophytes, r=-0.80, p<0.05; cystocarpic plants, r=-0.69, p<0.05; male gametophytes, r=-0.88, p<0.05). The lengths of infertile plants showed no significant differences between the two periods (t=0.85, p>0.05), with means of 23.3±3.2 and 21.6±4.3 cm for the rainy and dry seasons, respectively.

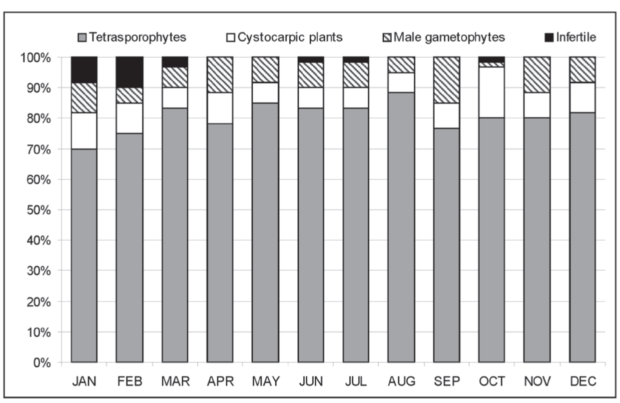

Figure 2. Frequencies of the different reproductive stages (tetrasporophytes, cystocarpic plants, and male gametophytes) and infertile plants of *G. birdiae* from Rio do Fogo Beach, Rio Grande do Norte State, Brazil, between January and December, 2008.

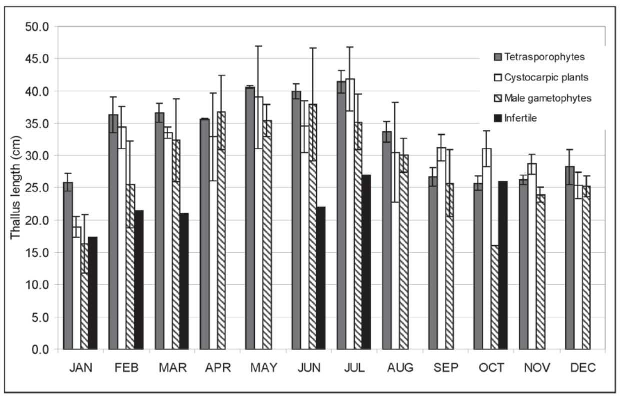

Figure 3. Mean thallus lengths for the different reproductive stages (tetrasporophytes, cystocarpic plants, and male gametophytes) and infertile plants of *G. birdiae* from Rio do Fogo Beach, Rio Grande do Norte State, Brazil, between January and December, 2008.

Discussion

Fertile plants of *G. birdiae* were found during the entire study period. The fertility of this population was evidenced by the small amount of infertile plants (~2%), which were recorded in only a few months of the year. Tetrasporophytes were found in larger amounts (80.1±5.6%), followed by cystocarpic plants (9.3±3.4%) and male gametophytes (8.3±3.6%). Dominance of the tetrasporophytic phase was also recorded in natural populations of various species of this genus, including *G. bursa-pastoris* and *G. coronopifolia* in Hawaii (Hoyle, 1978), *G. cornea* in Mexico (Orduña-Rojas & Robledo,

Table 2. Mean (±SD), minimum and maximum thallus length values (cm) of the different reproductive stages and infertile plants of *G. birdiae* from Rio do Fogo Beach, Rio Grande do Norte State, Brazil, during the rainy and dry seasons of 2008.

	Dry season		Rainy season		t-value	*p*
	Range	Mean±SD	Range	Mean±SD		
Tetrasporophytes	25.7-36.3	28.2±4.1	33.7-41.4	38.0±3.1	-8.05	0.000
Cistocarpic plants	18.9-34.3	28.3±5.5	30.5-41.8	35.4±4.2	-3.60	0.001
Male gametophyte	16.0-25.7	22.1±4.6	30.0-37.9	34.6±2.9	-4.52	0.000
Infertile plants	17.4-26.0	21.6±4.3	21.0-27.0	23.3±3.2	-0.86	0.413

2002), *G. damaecornis* in Venezuela (Brito & Silva, 2004), *G. gracilis* in Italy (Polifrone et al., 2006), and G. gracilis in Argentina (Martín et al., 2010). Studies suggest that tetrasporophytic dominance in nature may be related to the higher carpospore viability and survival rates as compared to the haploid stages (Destombe et al., 1989). According to Yokoya & Oliveira (1993), this dominance is due to the fact that diploid cells are supposedly more tolerant to environmental variations than haploid cells, which would explain the predominance of the tetrasporophytic phase in natural populations.

Over the course of the study, the sexual proportion of *G. birdiae* was 1:1 in most of the months studied, except February (♂1 : ♀2; $\chi 2 = 11.11$; $p<0.05$), September (♂1.8:♀1; $\chi^2=8.16$; $p<0.05$) and October (♂0.1 : ♀1; $\chi^2 = 66.94$; $p<0.05$). The significant increase in cystocarpic plants in October may be related to the greater proportion of male plants observed in the previous month ($p<0.06$). Earlier studies showed that inversion between the proportions of male and female plants may occur during peaks of sexual reproduction. These studies also underscore the fact that large amounts of cystocarpic plants commonly appear after a high frequency of male plants is recorded (Kain (Jones) & Destombe, 1995; Espinoza-Avalos, 2005). Infertile plants accounted for an insignificant portion of the population, compared to the other reproductive stages (tetrasporophytes and fertile gametophytes). However, their appearance was synchronized semi-monthly, supposedly after spore release, indicating the presence of juveniles from other reproductive stages.

In relation to thallus size, a clear seasonal variation was observed, with the highest values recorded in the rainy season and the lowest in the dry season. This type of seasonal model was also observed in other species of *Gracilaria* in tropical regions (Silva et al., 1987; Luhan, 1996; Orduña-Rojas & Robledo, 2002). During part of the rainy period (March-August), environmental conditions were characterized by waters rich in nutrients transported to the sea by the heavy rainfall. This supply of nutrients favored the luxuriant growth of *G. birdiae* until the month of August. The decreased thallus length from the month of August onward coincided with the increase in wind speed, waves, currents, and sediment displacement towards the seaweed banks. The combined effect of these factors between August and November contributed to the partial interment of the *G. birdiae* population and, in turn, to thallus fragmentation.

The effect of sediment load on seaweeds probably hindered thallus regeneration and contributed to the decreased recruitment, growth, and survival rate of the specimens. Eriksson & Johansson (2003, 2005) suggest that high sediment load is a negative factor for recruitment, growth, and fixation of spores. Thus, the marked presence of small infertile individuals in January, after hydrodynamic disturbances, indicates the restoration of the *G. birdiae* population and demonstrates the important effect of environmental conditions on the reproductive phenology of this population.

The results obtained in the present study provide relevant information about the reproductive aspects of a natural population of *G. birdiae*, located at Rio do Fogo Beach, in Northeastern Brazil. This knowledge is important for developing sustainable management and commercial exploitation programs for this natural resource.

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