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Reproductive morphology and phenological aspects of one morphological variant of *Hypnea pseudomusciformis* (Gigartinales, Rhodophyta)

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ABSTRACT

Hypnea pseudomusciformis was recently described from South America, and has three morphological variants: “musciformis”, “nigrescens”, and “valentiae”. Information on the biology of these variants may help to explain this species’ wide morphological variation despite the absence of genetic divergence among variants. More morphological and ecological data has accumulated on the “musciformis” variant occurring on the Brazilian coast than for the others. In this study, we described the reproductive morphology of a tropical “nigrescens” population and investigated its phenology to provide crucial biological information about this taxon, and perhaps also assist in answering questions about the systematics of *H. pseudomusciformis* variants. The population analyzed showed no significant fluctuations in its total biomass throughout the year. All reproductive stages were frequently recorded during this study, which contributes greatly to our knowledge of the reproductive morphology of the “nigrescens” variant. Phenological variations were correlated with environment variables, such as air and sea-surface temperatures, insolation, precipitation, and humidity. Male gametophytes were frequently present, which has never been reported for the “musciformis” variant. We showed that, despite being members of the same genetic species, the “nigrescens” and “musciformis” morphological variants exhibit remarkable differences in their ecology and biology.

Keywords: biomass, Cystocloniaceae, ecology, *Hypnea musciformis* complex, reproduction, systematics

Introduction

Species of the red algal genus *Hypnea* have the polysaccharide *kappa*-carrageenan as the main constituent of their cell walls, which is widely used in the food, cosmetic, and pharmacological industries (Knutsen *et al*. 1995; Masuda *et al*. 1997; Reis *et al*. 2008; Najam *et al*. 2010; Caires *et al*. 2013a). Carrageenan is used in the production of candy, tungsten filament coating, soups and sauces, creams, soaps, and insecticides, and also in the photographic, dairy, textile, and paper industries. Previous studies have shown that some *Hypnea* species have fungicidal (Melo *et al*. 1997) and anti-inflammatory

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(Bitencourt *et al*. 2008) properties, and can also be used as fertilizers in rice plantations (Selvan & Kannan 1990).

Hypnea pseudomusciformis is a species recently described from the South Atlantic Ocean, which is closely related to the previously described species *H. musciformis* from other parts of the world (Nauer *et al*. 2015). Based on molecular data, Nauer *et al*. (2015) determined that the species *H. pseudomusciformis* encompasses three morphological variants that were previously described from Brazil as distinct taxa, specifically *H. musciformis*, *H. nigrescens*, and *H. valentiae*. For decades, Brazilian authors (Schenkman 1986; Nunes 2005; Jesus *et al*. 2013b; 2014; Nauer *et al*. 2015) distinguished these taxa based on differences in their life habits and gross morphology as follows: the "musciformis" variant has abundant tendrils at the ends of its branches and branchlets; the "nigrescens" is easily recognized in the field by its epilithic habit, green or blackish color, and the resemblance of the appearance of their branches the bristles of a brush; and the "valentiae" has delicate thalli, reaches the greatest heights among all three variants, and has a more open and loosely arranged pattern of branching.

Research conducted on Brazilian *Hypnea* species has demonstrated that, in addition to the marked differences in their morphological traits, the morphological variants of *H. pseudomusciformis* also exhibit differences in their life habits, habitats, and reproduction (Schenkman 1986; Nunes 2005; Jesus 2012; 2016; Jesus *et al*. 2013b; 2014) that do not seem to be related to genetic differentiation among them (Nauer *et al*. 2015). Specimens with the "musciformis" morphology mainly forms sublittoral epiphytic populations, while the "nigrescens" variant usually grows attached to rocks in the middle intertidal zone on rocky shores with strong wave inputs, and the "valentiae" generally occurs in areas protected from direct wave action, such as in tidal pools. These variants rarely occur in sympatry and, when this occurs, one variety usually forms larger populations while the other(s) remain restricted to a few individuals each (personal observation).

Schenkman (1986) observed that morphological differences among these three variants are maintained in culture, demonstrating that these differences are determined by more than just different environments. Recently, Nauer *et al*. (2017b) carried out *in vitro* breeding tests between "nigrescens" and "musciformis" variants, which confirmed that these taxa belong to the same species according to the biological species concept (Wright 1940; Mayr 1942; Dobzhansky 1950). There is great disparity in the extent and types of information accumulated concerning different aspects of the biology and ecology of these morphological variants along the Brazilian coast. While many different aspects of the morphology, ecology, economical proprieties, and physiology of the "musciformis" variant have been well-studied (Schenkman 1986; 1989; Selvan & Kannan 1990; Knutsen et al. 1995; Wallner *et al*. 1992; Masuda *et al*. 1997; Melo *et al*. 1997; Reis & Yoneshigue-Valentin 2000; Nunes 2005; Bitencourt *et al*. 2008; Reis *et al*. 2008; Najam *et al*. 2010; Jesus 2012; 2016; Caires *et al*. 2013a; b; Jesus *et al*. 2013a; b; 2014; Nauer *et al*. 2015; among others), little or none information exists on the biology of the other variants of this species. It is crucial that essential biological information about these taxa be obtained to assist in the resolution of their intraspecific relationships.

Ecological traits are generally used to complement the descriptions of algal species; however, evaluations of variations in phenology are not commonly used at taxonomic scale in phycological studies. Espinoza-Avalos (2005) defined the phenology of macroalgae as the timing of recurring biological events, as well as the underlying causes of their timing by abiotic and biotic factors. Several studies have been conducted to describe the phenology and reproductive biology of *Hypnea* species (Mshigeni 1976a; b; c; Rao 1977; Rangaiah & Rao 1983; Schenkman 1989; Wallner *et al*. 1992; Reis & Yoneshigue-Valentin 2000; Reis *et al*. 2008; Smith *et al*. 2002; Kong & Ang Jr. 2004; Mouradi *et al*. 2008; Shameel *et al*. 2012; Caires *et al*. 2013b), and most have claimed that their phenology is strongly influenced by environmental factors. At the same time, Forrest & Miller-Rushing (2010) drew attention to the fact that phenology is an important adaptive trait resulting from a complex interplay among an organism's genes and several external environmental factors.

Thus far, phenological analyses of *Hypnea pseudomusciformis* along the Brazilian coast were only performed on the "musciformis" variant, and these studies observed the prevalence of tetrasporophytes and absence of male gametophytes throughout the year in the region (Schenkman 1989; Wallner *et al*. 1992; Reis & Yoneshigue-Valentin 2000; Caires *et al*. 2013b). Because of this, the reproductive morphology of the gametophytes of *H. pseudomusciformis* is poorly known, especially for male specimens, which may lead to misinterpretations in phenological studies. "valentiae" has a restricted distribution and forms small populations (Schenkman 1986; Jesus 2012; Nauer *et al*. 2015), making it difficult to assess its phenology. Conversely, "nigrescens" seems to be widely distributed along the Brazilian coast (Schenkman 1986; Nunes 2005; Guimarães 2006; Lyra *et al*. 2007; Jesus 2012; Jesus *et al*. 2013b; Nauer *et al*. 2015). In Brazil's Bahia state, "nigrescens" forms large populations on the north coast, although these occur on beaches that are very distant from each other.

In the present study, we described the reproductive morphology of the "nigrescens" variant of *H. pseudomusciformis* and evaluated its biomass and phenology in a tropical area of the Brazilian coast, including correlating variations in its phenology with changes in abiotic factors. This will provide essential biological and ecological information about this taxon, which can be used to help address systematic questions within the species *H. pseudomusciformis*.

Materials and methods

Study site

The present study was carried out on a rocky shore of Emissário Beach (12°44'25.70"S 38°8'58.97"W), located at Arembepe, on the north coast of Bahia State, Brazil (Fig. 1). The shore here is fragmented, directly exposed to strong hydrodynamic forces, and mainly occupied by sessile mollusks and calcareous algae. This site was chosen because of the presence there of uncommonly large beds of the "nigrescens" variant of the red macroalga *Hypnea pseudomusciformis* Nauer, Cassano *et* M.C. Oliveira. According to Leão *et al*. (2010), the region containing this site has a tropical climate, with precipitation of as much as 1300 mm y^{-1} in its northern portion. Average air temperatures range from 23 °C during the winter to 28 °C in the summer, and surface temperatures of the seawater vary from approximately 24 °C in winter to 28 °C in summer. Spring tides at the site can reach tidal ranges of up to 3.0 m.

Sampling

Samples were collected on a monthly basis from August 2013 to July 2014 using a destructive sampling methodology (Wreede 1985). Samples were collected in the intertidal zone during spring low tides (0.0 m to 0.3 m), and always during the mornings within the first fortnight of each month. For quantitative inferences (phenological and biomass estimates), three 20-m transects were established parallel to the shoreline. In each transect, five random points were selected at which 0.04 m^2 quadrats were examined. Each quadrat was considered as one sample, with a total of 180 samples analyzed (15 samples per collection). All specimens contained in the quadrats were collected. For analyses of frond lengths, individuals were arbitrarily collected from the area around each transect in each month, with approximately 20 specimens sampled per transect per month for a total of 250 specimens. All specimens were collected with the aid of a metal spatula, and transferred to the laboratory in plastic bags containing seawater.

Reproductive morphology

Samples were fixed in 4 % formalin and analyzed under a stereomicroscope (Leica Zoom® 2000) to remove algae epiphytes and sediment, and to identify the reproductive stages of the specimens in each sample. Detailed morphological analyses of each reproductive stage were conducted following Jesus (2012). Histological sections were made by hand with a razor blade, and reproductive structures were stained with 5 % aniline blue solution. Identification of structures was done under a stereomicroscope and a photonic microscope (Olympus® CBA) with an attached measuring ocular lens. Photomicrographs of reproductive structures were taken with the aid of an image capture program (QCapture Pro) and a digital camera (QImaging GO-3) attached to the photomicroscope (Olympus trinocular CX31RTS5®).

The following features of the reproductive morphology of the "nigrescens" variant were observed: (1) general appearance of the gametophyte/tetrasporophyte; (2) position of cystocarps on the axis, branches, and branchlets; (3) form and diameter of cystocarps; (4) presence of ostiole in cystocarps; (5) shape and diameter of carposporangia; (6) position of the spermatangial/tetrasporangial sori on the axis, branches and branchlets; (7) form and diameter of the spermatangial/tetrasporangial sori; (8) arrangement of spermatangia/tetrasporangia on fertile branchlets; and (9) shape and size of spermatangia/tetrasporangia.

Biomass and phenology

All collected specimens were separated into tetrasporophytes, female gametophytes (with cystocarps), male gametophytes (Fig. 2A-L), and non-reproductive (vegetative or infertile) fronds. Clumps of each specimen occur separately with stolon-like and erect branches arising a basal portion strongly anastomosed. After making phenological inferences, the seaweeds were dried in a drying oven (Fanem®, São Paulo, Brazil) at 60 °C for 48 h or until a constant weight was reached. Dry biomass data were then expressed as means standard deviation (*SD*) (g m^{-2}) or as percentages of total dry biomass. The lengths of fronds were measured from the base to the tip of each clump (i.e. specimen). After morphometric measurements were completed, these samples were herborized according to Nunes (2010) and deposited in the Herbarium Alexandre Leal Costa Bahia (ALCB) at Universidade Federal da Bahia (UFBA), with record numbers 114410 to 114475.

Abiotic factors

Daily means of data collected at 0h00min (12:00 am) for abiotic factors potentially affecting phenology, such as precipitation, humidity, monthly insolation, and air temperature, were calculated for use in analyses. These data were obtained from the National Meteorological Institute (INMET, http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep, accessed May 2018). Sea-surface temperature and salinity were measured *in situ* at the time of sampling as close as possible to the collection area using an electronic thermometer (model SH-102, JProlab, Parana, Brazil) and a portable refractometer (model 103, Biobrix®, São Paulo, Brazil), respectively.

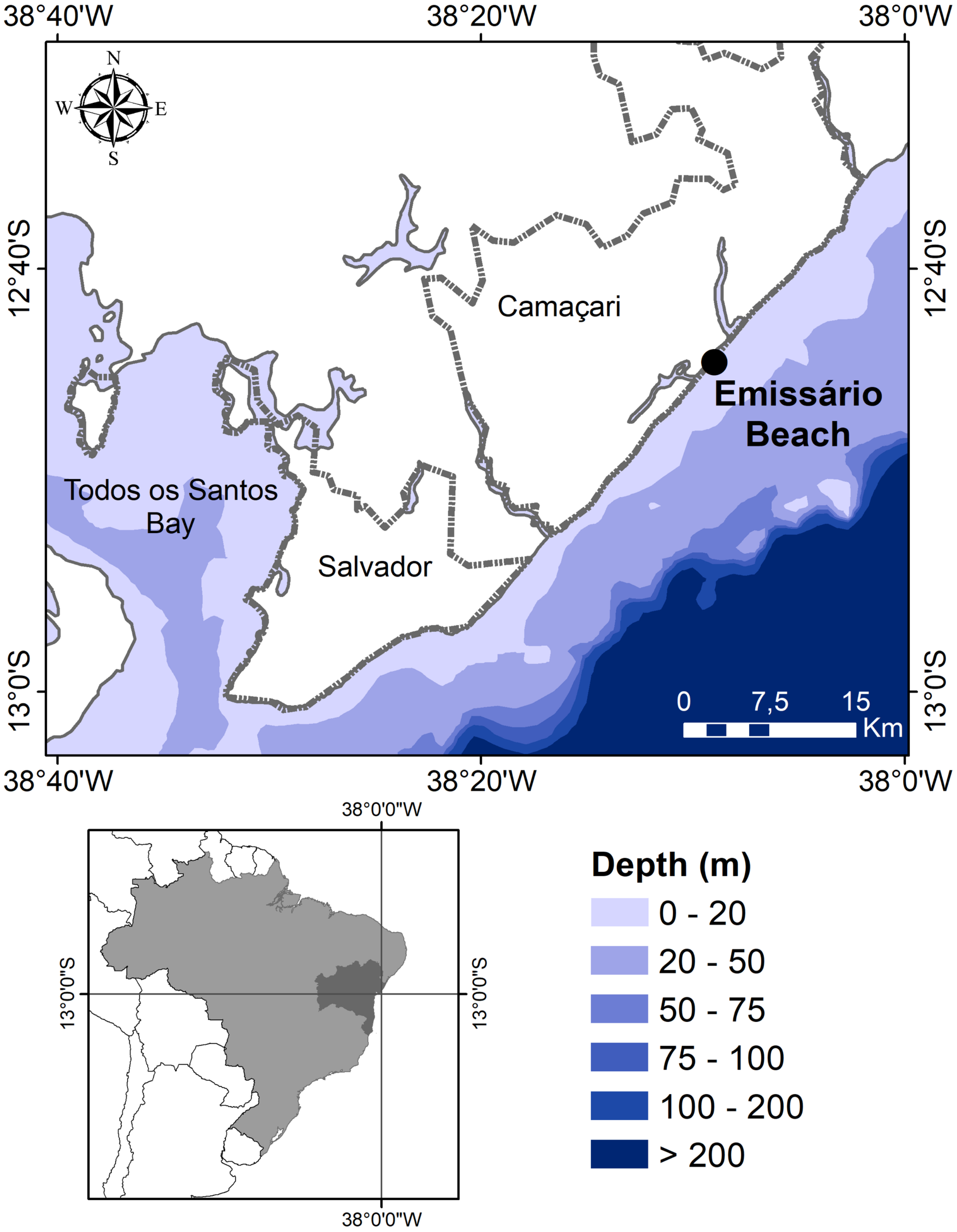

Figure 1. Map showing the study location at Emissário Beach, Bahia State, Brazil.

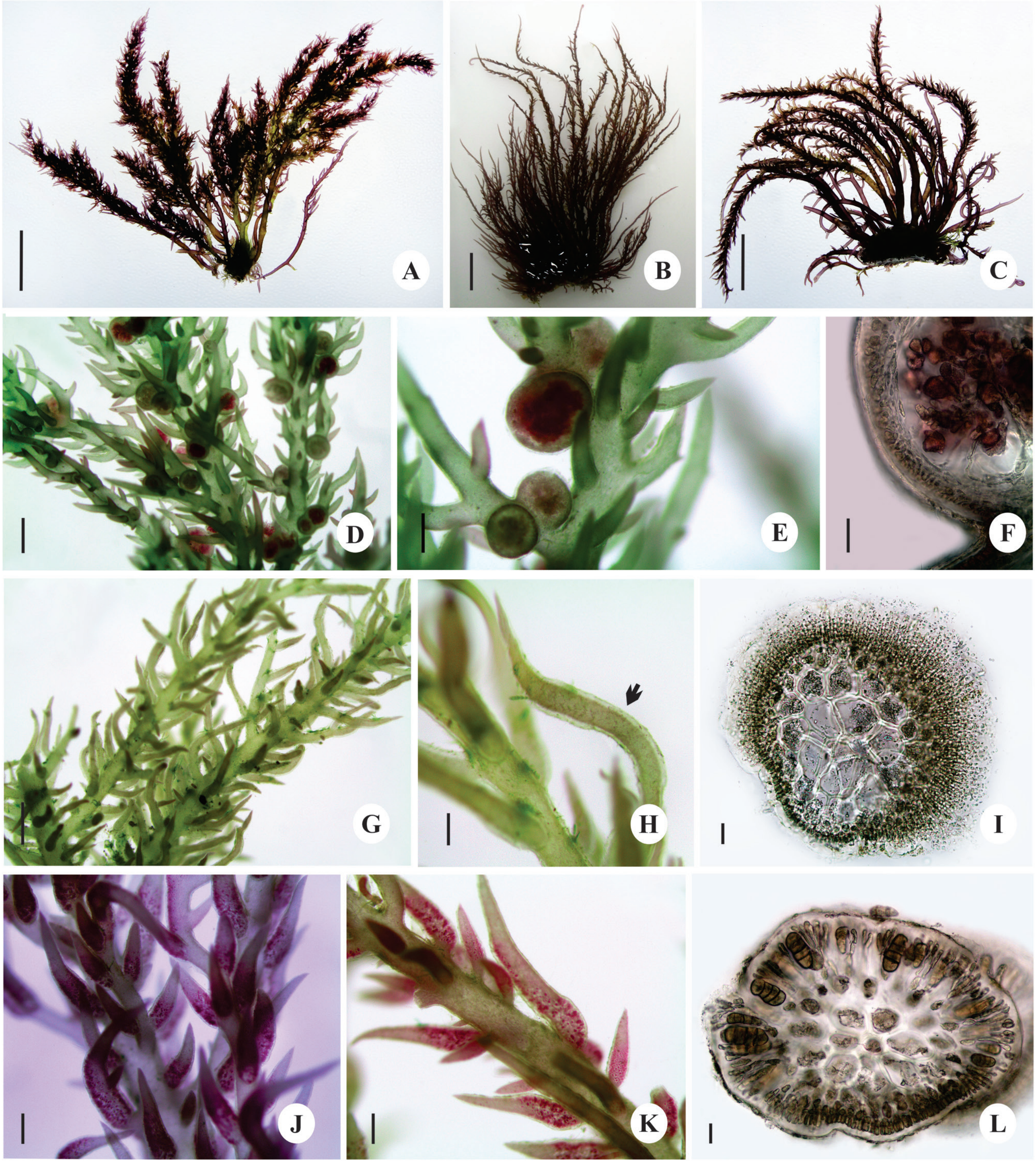

Figure 2. General appearance and reproductive morphology of the "nigrescens" morphological variant of *Hypnea pseudomusciformis*. **A.** General appearance of a female gametophyte plant. **B.** General appearance of a male gametophyte plant. **C.** General appearance of a tetrasporophyte. **D.** Cystocarps on branches and branchlets of a female plant. **E.** Cystocarps in detail. **F.** Longitudinal section through a mature cystocarp. **G.** Spermatangial sori in branchlets on a male plant. **H.** Magnified image of a spermatangial sorus (arrow). **I.** Cross-section of a branchlet incompletely surrounded by spermatangia. **J.** Tetrasporangial sori on branchlets. **K.** Magnified image of tetrasporangial sori. **L.** Cross-section of a branchlet incompletely surrounded by tetrasporangia. Scale bars: A, B and C = 1 cm; D = 500 μm; E = 600 μm; F = 40 μm; G = 1cm; H = 200 μm; I = 100 μm; J = 400 μm; K = 300 μm; L = 100 μm.

Statistical analyses

Data for dry biomass, phenology, and frond length were tested for normality of residuals and homoscedasticity with the Shapiro-Wilk and Levene's tests, respectively. The data were nonparametric; therefore, were normalized by logarithmization [log (x + 1)] and square root for biomass and length of fronds, respectively. We used a two-way ANOVA to verify if there were significant differences in the biomass and lengths of fronds, considering the fixed factors months and stages, in the study period. A Tukey's test was applied *a posteriori* to verify which periods showed significant differences throughout the study. We also performed Spearman's correlation tests to determine the possible relationships of the biomass and lengths of thalli with the environmental variables analyzed. For all analyses, the significance level was set at $p \leq 0.05$. All statistical analyses were carried out in an R software environment (R Core Team 2018).

Results

Reproductive morphology

The "nigrescens" variant was observed in this study to occur as epilithic thalli that are strongly attached to the substrate and form clumps. The thallus is erect, cartilaginous, green to greenish black, and measures 2.5–8 cm in height. Tufts are formed with a portion of the rhizoidal branches strongly adhered to the substrate, from which stolon-like and erect branches originate. Usually the longer axes are inserted at the central portion of the tuft, resembling the bristles of a brush. The apices of branches and branchlets are usually curved but may be straight. Subulate branchlets (each with a broad base and tapered apex) are irregularly arranged from 0.5 to 1.0 cm above the base, and are typically naked. The median and apical portions of the thallus are intensely and irregularly branched, with a tendency to spiral.

The appearance of the tetrasporic, female and male plants are shown in Figure 2A-C. Female plants (Fig. 2A) are more robust and display more intense branching than male (Fig. 2B) and tetrasporic (Fig. 2C) plants. Cystocarps are produced from the median to the apex of the axis and branches, occurring singly or in aggregations (Fig. 2D); they are globose (Fig. 2E), 357–599 µm in length and 393–601 µm in diameter, each have a thick pericarp and does not display evident ostioles (Fig. 2F). Carposporangia are ovate, 17–31 µm in diameter, and dispersed within the cystocarp. Spermatangial sori are produced from the median to the apex of the axis and branches (Fig. 2G), are more swollen and lighter in color than the sterile branchlets. Spermatangia incompletely surround the basal and median portion of the branchlets and make them curve and resemble a cloud around each branchlet (Fig. 2H); they are spherical, 4.1–6.3 µm in diameter, and arranged in a concatenated manner in the cortical region (Fig. 2I). Tetrasporangial sori are formed from the median to the apex of the axis and branches (Fig. 2J), and are more swollen and darkly pigmented than sterile and spermatangial areas (Fig. 2K). Zonate tetrasporangia are 36–167 µm in length and 15–74 µm in diameter (Fig. 2L), typically incompletely surround the basal and median portion of the branchlets, and make the branchlets curve.

Environment data

The abiotic data analyzed in this study showed values typical of a tropical region (Tab. 1). Mean air temperatures ranged from 23 °C in July 2014 to 26.4 °C in April 2014, and the total monthly insolation ranged from 147 h in August 2013 to 230 h in April 2014. The accumulated precipitation ranged from 41 mm in December 2013 to 167 mm in June 2014, whereas the average humidity raged from 81.4 % to 88 % in April and June 2014, respectively. Sea-surface temperatures varied by a maximum range of 4 °C (26 to 30 °C; 27 ± 1.4 °C), while the maximum variation in salinity was 9 (31 to 40; 36 ± 2.3).

Table 1. Environmental variables recorded on the coast of Bahia State, Brazil between August/2013 and July/2014.

Month	Accumulated precipitation (mm)*	Mean monthly humidity (%)*	Air temperature (°C)*	Total insolation (h)*	Sea surface temperature (°C)**	Salinity**
August/2013	100	84.8	27	186.1	30	37
September/2013	81.8	83.8	27.4	191.3	26	36
October/2013	79.6	82.4	28.2	205.1	26.6	36
November/2013	85.4	83.1	29	196.3	26	36
December/2013	41.0	82.8	29.3	167.5	26.9	35
January/2014	41.1	81.6	29.5	204.9	27.2	31
February/2014	42.2	83.8	29.4	188.3	27.7	33
March/2014	91.0	82.1	30	228.3	27.9	40
April/2014	47.7	83.8	29.2	200.7	26	37
May/2014	85.6	87.2	27.5	153.1	27.4	36
June/2014	166.8	87.2	26.7	148.9	28.9	34
July/2014	96.2	86	26.4	142.5	29.6	37

*Font: National Institute of Meteorology (INMET); **Measured in the study area.

Biomass, phenology, and frond length analyses

The “nigrescens” variant was present on Emissário Beach in all the months in which samples were collected in this study. The total monthly biomass of this alga ranged from 0.14 to 0.50 g m^{-2}, with no significant differences (p = 0.15) occurring among any of the months throughout the collection period (Tab. 2, Fig. 3). Tetrasporophytes and female gametophytes were found throughout the entire sampling period. Male gametophytes were absent in August 2013, while vegetative fronds were very rare in general, being observed only in August 2013. The biomass of male gametophytes throughout the year was low, and therefore the biomass of male gametophytes did not differ significantly among the months studied (Tab. 2). The biomass of tetrasporophytes, however, varied among the months studied. Specifically, the biomass of tetrasporophytes measured in June 2014 was significantly lower than that measured in October 2013 (p = 0.02) and November 2013 (p = 0.01), and significantly higher than that observed in April 2014 (p = 0.004). The biomass of female gametophytes in April 2014 was significantly higher than that measured in August 2013 (p = 0.02). The biomass of tetrasporophytes was significantly different from those of vegetative (p = 0.02), cystocarpic (p < 0.01), and male fronds (p < 0.01) (Tab. 2).

Table 2. Summary of two-way ANOVA results for the biomass and lengths of fronds of the “nigrescens” population on Emissário Beach, Bahia State, Brazil among the months studied.

Biomass	F value	*p*
Months	2.652	0.15
Stages	84.464	0.001
Months:Stages	0.886	0.003
	min-max (mean ± SD)	
Tetrasporophytes	0.21 - 0.93 (0.58 ± 0.21)	a
Female gametophyte	0.07 - 0.23 (0.13 ± 0.07)	b
Male	0 - 0.16 (0.06 ± 0.04)	b
Size of the thallus	**F value**	***p***
Months	13.936	0.001
Stages	0.768	0.513
Months:Stages	1.061	0.392
	min-max (mean ± SD)	
Tetrasporophytes	3.41 - 7 (5.44 ± 0.99)	a
Female gametophyte	3.89 - 6.91 (5.34 ± 0.8)	a
Male gametophyte	4 - 6.5 (5.16 ± 0.96)	a

max = maximum value; min = minimum value; SD = standard deviation; p = probability

The percentage of the sampled population’s biomass made up of tetrasporophytes, female and male gametophytes, and non-reproductive fronds in each month are shown in Figure 4. Tetrasporophytic fronds accounted for more than half of the total dry biomass in all studied months (74.63 %), with female and male gametophytes representing the remaining 17.10 % and 6.80 % of the biomass, respectively. Considering the entire sampling period, vegetative fronds were very rare (1.47 %), occurring only during August 2013. We observed the highest frequency of tetrasporophytes in November 2013 (88.79 %) and the lowest in January 2014 (62.82 %), whereas the occurrence of cystocarpic fronds was lower in October 2013 (7.20 %) and higher in January 2014 (29.85 %). April 2014 was the month with the highest frequency of male fronds (13.95 %).

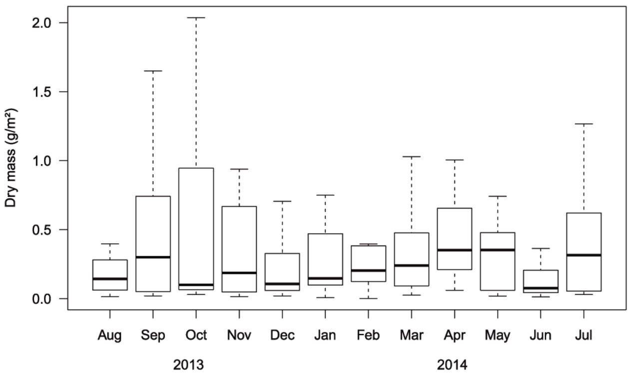

Figure 3. Total biomass (dry mass) of the “nigrescens” variant at Emissário Beach during the sampling period. Vertical lines = range of measurements; central boxes = inter-quartile ranges; central horizontal lines = median values.

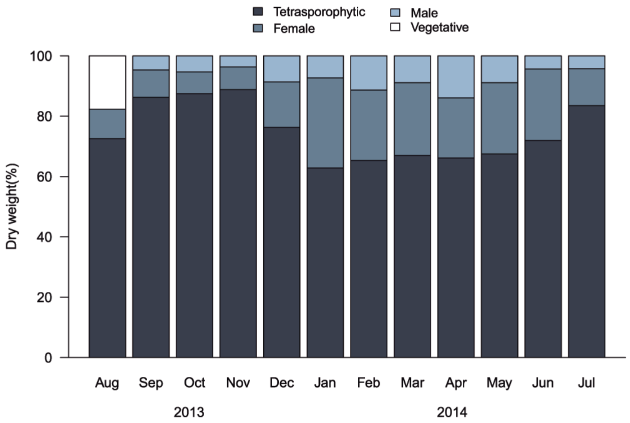

Figure 4. Percentages of biomass (dry mass) made up by each reproductive stage (vegetative, tetrasporophytic, and female and male gametophytes) of the “nigrescens” variant.

The sizes of thalli ranged from 3.64 cm to 6.77 cm (Tab. 2). Significant differences (p < 0.001) in frond lengths were detected throughout the sampling period. The smallest fronds were recorded in June 2014 (Tab. 2, Fig. 5). With respect to the mean thallus lengths of the different reproductive stages (Fig. 6), tetrasporophytes and female gametophytes reached their maxima in October 2013 and minima in June 2014, while male gametophytes reached their greatest lengths during February 2014 and their shortest lengths in June 2014.

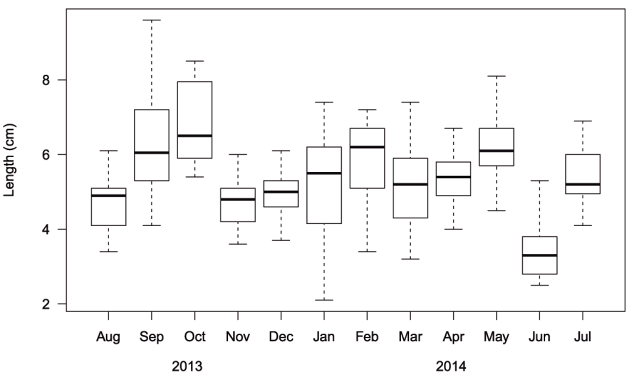

Figure 5. Variation in mean frond lengths of the "nigrescens" variant during the sampling period. Vertical lines = range of measurements; central boxes = inter-quartile ranges; central horizontal lines = median values.

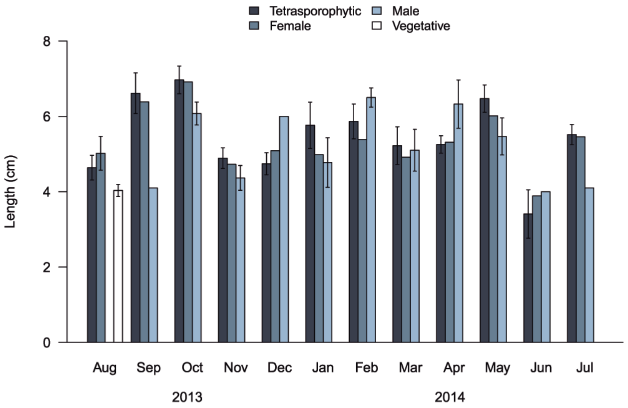

Figure 6. Mean thallus lengths for the different stages (vegetative, tetrasporophytic, and female and male gametophytes) of the "nigrescens" variant. Error bars represent standard errors.

Significant correlations between the biomass and sizes of fronds of the "nigrescens" variant and the environmental variables recorded during the study are shown in Table 3. Total biomass was negatively correlated with sea-surface temperature. A negative correlation was observed between the biomass of tetrasporophytes and sea-surface temperature. The biomasses of male and female gametophytes were positively correlated with air temperature and negatively correlated with relative humidity. Male gametophyte biomass was positively correlated with monthly insolation. Regarding frond lengths, only those of male gametophytes had any significant correlations with the environmental variables recorded; these were positively correlated with air temperature and monthly insolation, and negatively correlated with precipitation and relative humidity.

Discussion

In this study, we provided detailed information that can facilitate the detection of the occurrence of male gametophytes of the "nigrescens" morphological variant of *Hypnea pseudomusciformis*, which can be difficult to find and identify, especially in the field. A distinctive feature of this variant is that the tetrasporangia and spermatangia typically occur only on one side of the branchlets, causing a slight curvature in branchlets. The morphology of the gametophytes of *H. pseudomusciformis* is usually poorly described, with only their external appearance being emphasized. Schenkman (1986) assigned the difficulty in detecting male specimens, in part, to the absence of descriptions of these stages and their structures in bibliographic references. Contrary to this, recent taxonomic studies (Jesus *et al*. 2013a; b; 2014; 2015; 2016) have demonstrated the occurrence of male gametophytes in several *Hypnea* species, confirming that this reproductive stage is much more common than was previously reported.

The total biomass of "nigrescens" did not undergo significant fluctuations throughout the year, which differed from observations reported for the "musciformis" variant on the Brazilian coast (Schenkman 1989; Wallner *et al*. 1992; Reis & Yoneshigue-Valentin 1998; Faccini & Berchez 2000; Caires *et al*. 2013a). Reis & Yoneshigue-Valentin (1998) related these fluctuations in the biomass of "musciformis" to environmental factors, although they also pointed out the absence of such a seasonal pattern in populations from Rio de Janeiro State. On the other hand, data from previous published studies carried out on the coast of Bahia state showed conflicting patterns: Wallner *et al*. (1992) reported decreasing biomass during the summer, whereas Caires *et al*. (2013a) recorded the highest biomass values during this season. We observed a decline in the total biomass of "nigrescens" plants in the winter (June 2014), when the highest accumulated precipitation was recorded; however, no statistically significant differences among months were evident in the present study.

Table 3. Significant correlations between biomass and frond lengths of the "nigrescens" variant and various environmental variables measured at Emissário Beach, Bahia State, Brazil, from August 2013 to July 2014.

	Environmental variables	*r* value	*p* value
Biomass			
Total	Sea surface temperature	-0.69	0.01
Tetrasporophyte	Sea surface temperature	-0.65	0.02
Female gametophyte	Air temperature	0.76	0.004
	Relative humidity	- 0.69	0.01
Male gametophyte	Air temperature	0.78	0.004
	Insolation	0.67	0.02
	Relative humidity	- 0.76	0.01
Total frond length			
Male gametophyte	Air temperature	0.70	0.01
	Insolation	0.82	0.002
	Precipitation	-0.65	0.03
	Relative humidity	-0.84	0.001

r = Spearman's rho correlation coefficient; *p* = probability

Regarding the reproductive phenology of the "nigrescens" population studied herein, the highest proportion of the total biomass was observed to be made up by tetrasporophytes, followed by female and male gametophytes, and then by vegetative fronds. According to Cecere *et al*. (2000), the dominance of populations by the tetrasporophyte appears to be very common in red algae, and this was also previously reported in natural populations of *Hypnea* species (Rangaiah & Rao 1983; Schenkman 1989; Wallner *et al*. 1992; Faccini & Berchez 2000; Reis & Yoneshigue-Valentin 2000; Kong & Ang Jr. 2004; Mouradi *et al*. 2008; Caires *et al*. 2013b). Two main hypotheses can explain the predominant occurrence of the tetrasporophytic phase in *Hypnea* populations: (1) tetrasporophytes may produce more upright thalli per plant and are thus more robust under stressful conditions (Mathieson 1989); or (2) populations maintain themselves mainly by vegetative reproduction (Schenkman 1989). The latter hypothesis is possibly the most plausible for the studied population because it developed under conditions of strong and frequent water motion, thereby resulting in fragmentation of the fronds and their regeneration as diploid thalli.

Female and male gametophytes of the "nigrescens" variant were frequently found throughout the sampling period. This observation contradicts those of most previous studies of the "musciformis" variant in Brazil (Schenkman 1989; Wallner *et al*. 1992; Reis & Yoneshigue-Valentin 2000; Caires *et al*. 2013b), and those of other *Hypnea* species throughout the world (Rao 1977; Rangaiah & Rao 1983; Kong & Ang Jr. 2004; Mouradi *et al*. 2008), in which gametophytes were reported to be rare or absent. Already the vegetative fronds were found only in August 2013, despite representing a considerable proportion (~20 %) of the total biomass collected during this month. Non-fertile specimens of other *Hypnea* species have been regularly reported, and their presence is attributed to increases in conditions stressful for plant growth caused by abiotic factors (Reis & Yoneshigue-Valentin 2000).The rare incidence of vegetative specimens in the present study, as well as the frequency of female and male gametophytes, could be an indication that this population grows in a favorable environment despite experiencing high hydrodynamic activity, and the algae therein are thereby able to complete their full life cycle and reproduce sexually.

The total biomass of the studied "nigrescens" population, as well as the biomass of tetrasporophytes, was significantly and negatively correlated with sea-surface temperature. This was also observed for the "musciformis" variant in southeastern and northeastern Brazil (Schenkman 1989; Wallner *et al*. 1992), and in other tropical and subtropical populations of *Hypnea musciformis* in several locations across the world (Rao 1970; Guist Jr *et al*. 1982; Kong & Ang Jr. 2004). Mshigeni (1977) inferred that the high-light intensity and high-temperature conditions of the summer were among the most important factors responsible for the reduction of *Hypnea* populations they observed during this season. Despite this, temperate populations appear to show a different trend, with the biomass of this alga generally reaching a maximum during the summer (Friedlander & Zelikovitch 1984; Aziza *et al*. 2008). Reis & Yoneshigue-Valentin (2000) and Caires *et al*. (2013b) reported the presence of cystocarpic plants in "musciformis" only during the summer. Wallner *et al*. (1992) interpreted this as a response by the alga to improved light conditions. Despite of the biomass of female gametophytes showed no correlation with insolation, in the present study, we observed a positive correlation between both (female and male) gametophytes biomass with air temperature, and a negative correlation between with relative humidity, indicating that these conditions (usually observed in the Brazilian summer) could be related to the induction of fertilization and consequent development of cystocarps.

We observed significantly shorter thalli in June 2014, which coincided with the highest values of accumulated precipitation and humidity, and low values of air temperature and insolation. These factors, in conjunction with the strong waves observed at the collection site, could have contributed to thallus fragmentation (Reis & Yoneshigue-Valentin 2000; Kong & Ang Jr. 2004), and hence to the decreased lengths of thalli observed. The lengths of the thalli of tetrasporophytes and female gametophytes were not correlated with any of the environment variables recorded. Despite this, the lengths of male gametophytes were positively correlated with air temperature and insolation, and negatively correlated with precipitation and humidity, confirming that the optimal conditions for the development of this stage occurred during the dry season. Cecere *et al*. (2000) postulated that gametophytes are ephemeral; therefore, they could die soon after reproduction or become reproductive when still so small as to be undetected. Our findings suggested that, at least for the studied population of the "nigrescens" variant of *Hypnea pseudomusciformis*, this is not true, since tetrasporophytes and gametophytes had similar thallus heights. Nauer *et al*. (2017a) demonstrated *in vitro* that "musciformis" has a higher growth rate than "nigrescens", which could explain some of the differences observed and should be evaluated in the field.

Our results provide important data on the reproductive biology of a neglected intraspecific taxon, and provide evidence of the potential influence of the environmental variables we analyzed on its phenology. We have shown that the variant "nigrescens" exhibits remarkable differences in its phenology from the "musciformis" variant, especially regarding the expression of its sexual phases. Despite being recently combined into a single genetic species together with the "valentiae" variant (*Hypnea pseudomusciformis*), these taxa appear to present not only morphological differences, but also behavioural, developmental, and ecological differences from one another. Recognizing the factors that determine all of these differences remains a major challenge to the resolution of these infraspecific relationships, which needs to be investigated through further research, especially since the morphological varieties are not yet formally proposed taxa.

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