Latitudinal variation in phlorotannin contents from Southwestern Atlantic brown seaweeds

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Phlorotannins are primary and/or secondary metabolites found exclusively in brown seaweeds, but their geographic distribution and abundance dynamic are not very well understood. In this study we evaluated the phlorotannin concentrations among and within-species of brown seaweeds in a broad latitudinal context (range of 21°) along the Brazilian coast (Southwestern Atlantic), using the Folin-Ciocalteau (FC) colorimetric method. In almost all species (16 out of 17) very low phlorotannin concentrations were found (< 2.0%, dry weight for the species), confirming reports of the typical amounts of these chemicals in tropical brown seaweeds, but with significantly distinct values among 7 different and probably highly structured populations. In all 17 seaweed species (but a total of 25 populations) analyzed there were significant differences on the amount of phlorotannins in different individuals (t-test, p < 0.01), with coefficients of variation (CV) ranging from 5.2 to 65.3%. The CV, but not the total amount of phlorotannins, was significantly correlated with latitude, and higher values of both these variables were found in brown seaweeds collected at higher latitudes. These results suggest that brown seaweeds from higher latitudes can produce phlorotannins in a wider range of amounts and probably as response to environmental variables or stimuli, compared to low latitude algae.
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Abstract
Phlorotannins are primary and/or secondary metabolites found exclusively in brown seaweeds, but their geographic distribution and abundance dynamics are not very well understood. In this study, we evaluated phlorotannin concentrations among and within species of brown seaweeds across a broad latitudinal context (range of 21°) along the Brazilian coast (Southwestern Atlantic), using the Folin-Ciocalteau (FC) colorimetric method. We found very low phlorotannin concentrations (typically < 2.0% dry weight) in almost all species (16 out of 17), confirming reported concentrations in tropical brown seaweeds, but with significant differences among seven different and probably highly structured populations. In all 17 seaweed species analyzed (representing a total of 25 populations), we found significant differences in the amount of phlorotannins in different individuals (t-test, p < 0.01), with coefficients of variation (CV) ranging from 5.2 to 65.3%. The CV, but not the total amount of phlorotannins, was significantly correlated with latitude, and higher values for both these variables were found in brown seaweeds collected at higher latitudes. Our results suggest that brown seaweeds from higher latitudes can produce phlorotannins in a wider range of concentrations relative to those at low latitudes, probably in response to environmental stimuli.

Introduction
Phlorotannins are polymers derived from a simple monomer, phloroglucinol, found exclusively in brown seaweeds (Targett & Arnold 1998 2001). These water-soluble secondary metabolites constitute a special class of polyphenols that may exhibit multifunctional ecological roles, acting as a herbivore deterrent (Pereira & Yoneshigue-Valentin 1999), antifouling agent (Plouguerné et al. 2012), antioxidant (Crucès et al. 2012), UV protector (Henry & Van Alstyne 2004), and a chelating agent of toxic heavy metal ions (Karez & Pereira 1995). However, these chemicals may also be classified as primary metabolites when they are structural components of cell walls (Schoenwaelder & Clayton 1999). In fact, phlorotannins found inside the cells of brown seaweeds are stored in small vesicles called physodes, and these chemicals may exude into the environment due to their water solubility (Jennings & Steinberg 1994) where they can have several vital ecological roles (e.g. Pereira et al. 1990). As cell wall components, where
they form a complex with alginic acid, they are insoluble (Schoenwaelder 2002, Koivikko et al. 2005). Given the smaller amounts of cell-wall-bound phlorotannins compared to soluble phlorotannins, the major function of these chemicals appears to be secondary metabolites (Koivikko et al. 2005).

The concentration of phlorotannins in brown seaweeds is known to be highly variable in several modes and at various scales, supposedly in response to the dynamics of biotic and abiotic environmental conditions (Jormalainen et al. 2003). For example, concentrations may vary in response to environmental factors, either biotic – such as herbivory (Hemmi et al. 2004) and epibiosis (Plouguerné et al. 2010) – or abiotic – such as temperature (Cruces et al. 2012), irradiance (Cruces et al. 2013), nitrogen concentrations (Pavia & Toth 2000), bathymetric variation, and immersion time in the intertidal range (Connan et al. 2004). Phlorotannin content can also vary according to intrinsic aspects of brown seaweeds, such as individual size and age (Pavia et al. 2003), and tissue type (Plouguerné et al. 2012).

Another interesting aspect relating to the distribution, abundance, and function of phlorotannins is the latitudinal differences in content of these chemicals among brown seaweeds living along large temperate-tropical gradients (Steinberg 1989, Van Alstyne & Paul 1990). High concentrations of these compounds have been found in species from high latitudes (Ragan & Glombitza 1986, Steinberg & Paul 1990, Steinberg & Van Altena 1992, Hay & Steinberg 1992, Steinberg 1992). For example, species of Fucales and Laminariales that are abundant in temperate benthic communities, and Dictyota species found both in temperate and tropical regions, exhibit this biogeographic trend. The most common brown seaweed species in temperate Australasia exhibit more than 10% of total phlorotannins (Steinberg 1989), whereas there are both phlorotannin-rich and -poor species in some temperate regions of South Africa (Anderson & Velimirov 1982, Tugwell & Branch 1989), northwestern Pacific (Katayama 1951, Estes & Steinberg 1988), and the European North Atlantic (Ragan & Glombitza 1986).

Many species of brown seaweeds from North America exhibit low levels of phlorotannins, ranging from 0 to 2% of algal dry weight (Ragan & Glombitza 1986). This range is found mainly in kelps dominating both the sublittoral and lower littoral environments (Steinberg 1992). In contrast, as the most abundant organisms found in littoral and upper sublittoral regions, fucoids commonly contain higher phlorotannin contents (more than 4% dry weight) (Steinberg 1985, Van Alstyne 1988, Denton et al. 1990, Targrett et al. 1992). In general, brown seaweeds from North America exhibit broad variation in phlorotannin contents linked to the bathymetric gradient, with littoral fucoids and subtidal kelps showing high and low levels of these compounds, respectively (Estes & Steinberg 1988, Steinberg 1992).

In general, the intensity of selective pressures on organisms increases with decreasing latitude, including higher herbivory and epibiosis (Railkin 2004, Targrett & Arnold 1998). Consequently, tropical seaweeds are hypothesized to have evolved more effective chemical defenses (Van Alstyne & Paul 1990, Targrett et al. 1992). Contrary to this trend, phlorotannins are sometimes absent or present in very low concentrations in seaweeds from tropical environments (Steinberg 1989, Van Alstyne & Paul 1990, Pereira & Yoneshigue-Valentin 1999). There is only one report of high amounts of these compounds in brown seaweeds from low latitudes (Targrett et al. 1995).

However, in almost all studies, the quantification of phlorotannins is based on an analysis of distinct specimens of brown seaweed species extracted together, masking possible variability in amounts of these chemicals in each individual of a population. However, intra-populational variation in seaweed-derived chemicals can be of great magnitude and ecological significance (Oliveira et al. 2013).

Along the Brazilian coast, the few studies on phlorotannin contents in brown seaweeds are united in the fact that they typically reveal low concentrations (Fleury et al. 1994), and that they may be capable of inhibiting grazing when they occur at higher concentrations (Pereira & Yoneshigue 1999). The extensive Brazilian coast covers a broad latitudinal range of the Southwestern Atlantic and harbors numerous species of brown seaweeds. It comprises several environments suitable for exploring chemical defenses via a biogeographic approach. To date, most studies in Brazil have only reported average phlorotannin concentrations, so there is no information concerning the variation within populations or among populations from different latitudes. Thus, more in-depth analysis is needed, as tropical species could have the same mean value as temperate seaweeds, but exhibit greater standard deviation. Here, we hypothesized that contents of brown seaweed phlorotannins would exhibit latitudinal variation along the Brazilian coast. Our aim was to compare the mean phlorotannin concentration, as well as the coefficient of variation, among
and within species of brown seaweeds across a broad latitudinal context along the Brazilian coast to evaluate the hypothesis that species from low latitudes exhibit lower amounts of these chemicals relative to those from high latitudes.

**Materials & Methods**

**Study organisms and collection localities**

Brown seaweeds were collected from along the Brazilian coast (Instituto Chico Mendes de Conservação da Biodiversidade - Authorization Number 27001-2) in order to best represent various populations of the same species and individuals in each population from the different localities (Fig. 1, Table 1): Giz Beach (6°10’ S; 35°05’ W) at Tibau do Sul, RN; Itapuama (08°17’ S; 34°57’ W), Calhetas (08°20’ S; 34°56’ W), Paraiso (08°21’ S; 34°57’ W) and Suape beaches (08°22’ S; 34°56’ W) at Recife, PE; Itapuã Beach (12°57’ S; 38°22’ W) at Salvador, BA; Pé de Serra Beach (14°28’ S; 39°01’ W) at Uruçuca, BA; Morro de Pernambuco (14°48’ S; 39°01’ W) and Back Door beaches (14°56’ S; 39°00’ W) at Ilhéus, BA; Ponta Beach (16°24’ S; 39°02’ W) at Porto Seguro, BA; Três Praias (20°38’ S; 40°28’ W) at Guarapari, ES; Rasa (22°44’ S; 41°57’ W) and Forno beaches (22°45’ S; 41°52’ W) at Armação dos Búzios, RJ; and Canasvieiras Beach (27°25’ S; 48°28’ W) at Florianópolis, SC. We collected individuals of the following species: *Canistrocarpus cervicornis* (Kützing) De Paula & De Clerck, *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier, *Dictyopteris delicatula* J.V. Lamouroux, *D. polypodioides* (A.P. De Candolle) J.V. Lamouroux, *Dictyota ciliolata* Sonder ex Kützing, *D. crispata* J.V. Lamouroux, *D. dichotoma* (Hudson) J.V. Lamouroux, *D. mertensii* (Martius) Kützing, *D. pfaffii* Schnetter, *Lobophora variegata* (J.V. Lamouroux) Womersley ex E.C. Oliveira, *Padina gymnospora* (Kützing) Sonder, *Sargassum filipendula* C. Agardh, *S. stenophyllum* Martius, *S. ramifolium* Kützing, *S. vulgare* C. Agardh, *S. vulgare* var. *nanum* E. De Paula, *S. vulgare* var. vulgare, *Spatoglossum schoederi* (C. Agardh) Kützing, and *Stypopodium zonale* (J.V. Lamouroux) Papenfuss.

**Extraction**

After collection, the seaweeds were freeze-dried, ground to powder and, before extraction, subjected to a lipid-removal treatment using 1 mL hexane for 3 min (Koivikko et al. 2007). Extraction was then carried out for 2 h using 10 ml of acetone:water (7:3) for 100 mg of each sample of dry alga. Each extract was centrifuged for 10 min at 3500 rpm and filtered. Acetone was evaporated off at room temperature and the aqueous extract was again centrifuged. The supernatant was frozen for further quantification.

**Phlorotannin quantification**

We used the Folin-Ciocalteau (FC) colorimetric method to quantify phlorotannin concentration, by which 1 N FC reagent was added to a diluted aliquot of the extract and, after 3 min, 20% sodium carbonate was added. After 45 min in the dark, phlorotannins were quantified in a Shimadzu UV1800 spectrophotometer, at 750 nm, using a standard curve obtained with phloroglucinol (r² = 0.99), which is a monomer that absorbs under the same patterns as the polymers (phlorotannins) derived from it (Steinberg 1988). Three aliquots of each extract were prepared for quantification, and the total phlorotannin concentration is expressed in % per dry weight (DW) of the seaweed.

**Statistical analysis**

The coefficient of variation was calculated as the ratio of the standard deviation to the mean (CV = δ/µ.100) in order to compare the amount of variation in phlorotannin contents observed within different populations of seaweeds. Total phlorotannin content of different populations from the same species was assessed by independent t test or, when n was unequal, with an independent t test with separate variances, which is more appropriate when considering groups of different sample sizes. In the case of more than two populations from the same species, we conducted a unifactorial ANOVA followed by the post-hoc Student Newman-Keuls test (SNK).

**Results**

**Amounts of phlorotannins and their inter-populational variability**

Total phlorotannins ranged from 0.05 to 4.30% (average ± standard deviation) for the 17 brown seaweed species we studied (dry weight), encompassing a total of 25 populations (Table 2).
Variation in phlorotannin contents within populations and across a latitudinal gradient

Intra-populational analyses were carried out for 25 populations (Table 2) of 14 seaweed species (Table 1). For all analyzed populations, we identified a significant difference in the amount of phlorotannins among the individuals that comprised them (t test, p < 0.01), with coefficients of variation (CV) ranging from 5.2% to 65.3% (Table 2). CV was higher in populations collected from higher latitudes, but the correlation though significant (p < 0.005) was relatively weak (r = 0.55) (Fig. 2).
We assessed phlorotannin contents in brown seaweeds sampled along a broad latitudinal range, from 2° to 22° of southern latitude, representing from Recife to Rio de Janeiro, respectively (Table 2). The highest phlorotannin contents were found in brown seaweeds collected at higher latitudes, but the correlation between amounts and latitude was weak and non-significant (Fig. 3, $r = 0.23; p = 0.15$).

**Discussion**

The phlorotannin contents found in the brown seaweeds we investigated were typically very low (< 2.0% dry weight, DW), with only one exception, *Spatoglossum Schroederi* for which we recorded 4.30% DW. These results reinforce a pattern that seems to be typical of tropical areas, including the Brazilian coast, in which low values of phlorotannins have been reported for several brown seaweeds belonging to different orders, ranging from 0.2 to 2.17% DW (*e.g.* Pereira & Yoneshigue 1999, Pereira et al. 1990, Fleury et al. 1994). Low contents of these chemicals, varying from 0.19 to 1.62% DW, were also found in some brown seaweeds from Guam and neighboring areas of the tropical Pacific (Steinberg & Paul 1990, Van Alstyne & Paul 1990). Moreover, low levels of phlorotannins (ranging from 0.2 to 1.77 % DW) have been found in *Sargassum spp.* and *Turbinaria spp.* at two tropical sites, Tahiti and the Great Barrier Reef, Australia, respectively (Steinberg 1986).

Brown seaweed phlorotannins have been reported as defensive chemicals against herbivores in some studies (*e.g.* Jormalainen & Ramsay 2009), but only when they occur at concentrations higher than 2.0% DW, i.e. levels commonly found in species from temperate regions (Ragan & Glombitza 1986). However, the evidence for this defensive property of phlorotannins remains disputed, with reports supporting (Van Alstyne & Paul 1990) and refuting (Steinberg & Paul 1990) this role. The low levels of phlorotannins in tropical seaweeds may be due to these chemicals having limited impact on tropical fish herbivory, given that fishes from the Great Barrier Reef do not consume more phenolic-poor tropical species than phenolic-rich species (Steinberg & Paul 1990). However, contradicting this latter finding, phlorotannin-rich seaweeds were not consumed by fishes in Guam (tropical Pacific region), though extracts from phlorotannin-poor species were also not eaten (Van Alstyne & Paul 1990). Moreover, phlorotannins in amounts higher than those usually found in the Brazilian brown seaweed *Sargassum Furcatum* can inhibit herbivory (Pereira & Yoneshigue 1999). However, according to our results, almost all of the seaweeds we studied probably do not employ this kind of chemical defense to prevent herbivory, since phlorotannin contents were usually lower than 2.0% DW.

The hypothesis of a latitudinal gradient of phlorotannin contents is based on the assumption that herbivory pressure increases with decreasing latitude and that production of seaweed chemical defenses is selected by the action of herbivores. Accordingly, defensive chemicals should be more common and effective in tropical seaweeds. Although chemical defenses are commonly associated with herbivore abundance and pressure, no study has conclusively demonstrated that herbivores impose selective pressures on the production of secondary metabolites (*Van Alstyne & Paul 1990*). Moreover, phlorotannins may be present in brown seaweeds for reasons other than herbivore defense, since they have been suggested to exhibit other ecological roles, such as protecting against short-wave UV radiation (Pavia et al. 1997), and as anti-fouling agents (Plouguerné et al. 2010, 2012).

It would be difficult to establish a clear correlation between the latitudinal variability in phlorotannin production by brown seaweeds solely with the different pressures of herbivory along the Brazilian coast, even knowing that this kind of variation exists and that the seaweeds we studied were collected from a broad latitudinal range (ca. 21°). Importantly, it remains controversial if herbivory pressure selects for chemical defense production (*Pereira & Da Gama 2008*), even across a global tropical-temperate latitudinal gradient or along the Brazilian coast (*Longo et al. 2014*). In addition, it is known that concentrations of secondary metabolites may vary according to temperature (*Sudatti et al. 2011*), nutrient availability (*Puglisi & Paul 1997*), light (*Pavia et al. 1997*), salinity (*Kamiya et al. 2010, Sudatti et al. 2011*), and herbivory (*Weidner et al. 2004*). Thus, since the seaweeds we studied are also subjected to unknown variability in all these external conditions, it is perhaps not surprising that we did not establish a direct causal effect between phlorotannin content and latitude.

The extent of genetic control over chemical defense production remains poorly understood. For example, phlorotannin content was demonstrated to be due to genotypic variation in *Fucus vesiculosus* (*Jormalainen et al. 2003, Jormalainen & Honkanen 2008, Koivikko et al. 2008*), as well as for terpenes in the red seaweeds *Laurencia*
Temperature is a determining factor for the survival, geographic distribution, and reproduction of seaweeds (e.g. Padilla-Gamiño & Carpenter 2007), and it is also responsible for many responses of their primary metabolism, such as photosynthesis, growth (Nishihara et al. 2004), nutrient absorption (Tsai et al. 2005), and secondary metabolism (e.g. Sudatti et al. 2011). Thus, given the reduced gene flow known for seaweeds (e.g. Wright et al. 2000) and the different environmental conditions along the Brazilian littoral coast, populations of the same species we studied here could be highly structured, explaining in part the results we obtained. Accordingly, our field data reinforce the idea that genetic heterogeneity contributes to quantitative variation of secondary metabolism and that our sampled populations may represent ecotypes.

The intra-populational variability in the amounts of defensive chemicals we report here corroborates the findings of the few previous studies that investigated this topic in the red seaweeds *Porteria hornemannii* (Matlock et al. 1999), *Delisea pulchra* (Wright et al. 2000) and *Laurencia dendroidea* (Sudatti et al. 2006). However, those studies did not assess as broad a latitudinal context as we did. Our study also reinforces the importance of analysis at the intra-population level (i.e. variation among specimens), since most studies of seaweed chemical ecology overlook this element of chemical variation by examining pooled extracts and/or substances obtained from groups of individuals. Developmental (Bowers & Stamp 1993), environmental (Agrell et al. 2000), and genetic (Berenbaum & Zangerl 1992) traits all represent sources of variation that can explain the diversity of plant chemical phenotypes. Moreover, in seaweeds, life-history phases (see Vergés et al. 2008), ontogenetics (Paul & Van Alstyne 1988), and chemical races (Abe et al. 1999) may also be included as sources of secondary metabolite variability. In our analysis, the specimens belonged to the sporophytic life-history phase and were approximately of the same size. However, we cannot rule out the possibility that chemical races exist among the individuals of each population we studied.

### Conclusion

Overall, our results show that latitude does not explain the variability in total amounts of phlorotannins found in each population of the brown seaweeds we studied along the Brazilian coast, but the significant intra-specific differences in production of these chemicals we report may be important to understanding the ecological drivers of this defensive chemistry in seaweeds. Based on characteristics of the Brazilian coast (Floeter & Soares-Gomes 1999), the higher phlorotannin levels we recorded in populations from higher latitudes may represent a greater capacity for these seaweeds to respond to seasonal stimuli. Since environments in low latitudes exhibit little seasonal variation, the need for seaweeds in these zones to vary production of these chemicals may be lessened. Thus, brown seaweeds at higher latitudes are more likely to modulate chemical defense production in response to stimuli than those in tropical regions where the environmental conditions are more constant. However, we assert that further studies of intra-populational variability in chemical defense are warranted in the context of marine chemical ecology.

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Figure 1

Sampling sites

Sampling sites of collect of the brown seaweeds studied along the Brazilian littoral, in a latitudinal range of 21°.
Figure 2

Correlation: coefficient of variation (%) in content of phlorotannins and latitude

Correlation between the coefficient of variation (%) in content of phlorotannins found in brown seaweeds and latitude (sampling site of the seaweeds).

$r = 0.5535$
$r^2 = 0.3064$
$p = 0.0041$
Figure 3

Correlation: total content of phlorotannins x latitude.

Correlation between total content of phlorotannins found in the brown seaweeds and latitude (sampling site of the seaweeds).

\[ r = 0.2282, \quad r^2 = 0.0521, \quad \rho = 0.1513 \]
Table 1 (on next page)

Table 1

Brown seaweeds studied, number of specimens and corresponding collection places (x= The individuals were analyzed together because of the small size/biomass of the specimens; while in the remaining species, the analyzes were performed in each individual)
Table 1. Brown seaweeds studied, number of specimens and corresponding collection places (x=1. The individuals were analyzed together because of the small size/biomass of the specimens; while in the remaining species, the analyzes were performed in each individual)

| Seaweed species           | Sampling sites                      |
|---------------------------|-------------------------------------|
|                           | 1. Tibau do Sul – RN                |
|                           | 2. Recife - PE                      |
|                           | 3. Salvador - BA                    |
|                           | 4. Uruçua – BA                      |
|                           | 5. Ilhéus – BA                      |
|                           | 6. Porto Seguro – BA                |
|                           | 7. Guarapari - ES                   |
|                           | 8. Búzios – RJ                      |
|                           | 9. Florianópolis – SC               |
| Canistrocarpus cervicornis| 7 x                                 |
| Colpomenia sinuosa        | 4 x                                 |
| Dictyopteris deliciatula  | 8 10 x                              |
| Dictyopteris polypodioiides| x                                 |
| Dictyota ciliolata        | 11                                  |
| Dictyota crispata         | 19                                  |
| Dictyota dichotoma        | x                                   |
| Dictyota mertensii        | 7 x 10                               |
| Dictyota pfaffii          | 10                                  |
| Lobophora variegata       | 10 x 10                             |
| Padina gymnospora         | x 10 x 7 x 8                        |
| Sargassum filipendula     | 9 10 x 7                            |
| Sargassum ramifolium      | 7                                    |
| Sargassum stenophyllum    | 7                                    |
| Sargassum vulgare         | 15 6 10 22 5                        |
| Spatoglossum Schroederi   | 10                                   |
| Stylopodium zonale        | 32                                   |
Table 2

Table 2

Number of individuals (N), and mean total phlorotannin content (TPC) measured in % (average ± standard deviation) of dry weight (DW) for the populations of seaweeds studied from different collection sites, including the coordinates, coefficient of variation (CV) and the ANOVA results for intra-populational variation (IV).
Table 2. Number of individuals (N), and mean total phlorotannin content (TPC) measured in % (average ± standard deviation) of dry weight (DW) for the populations of seaweeds studied from different collection sites, including the coordinates, coefficient of variation (CV) and the ANOVA results for intra-populational variation (IV).

| Seaweeds         | Time of year | Location | Latitude (°S) | N  | TPC (%DW) | IV          | CV (%) |
|------------------|--------------|----------|---------------|----|-----------|-------------|--------|
| C. cervicornis   | Spring/09    | 2        | 8             | 7  | 0.13±0.01 | F = 45.3; p< 0.001 | 8.5    |
| C. cervicornis   | Summer/11    | 9        | 22            | +  | 0.18±0.00 | +           | +      |
| C. sinuosa      | Summer/11    | 7        | 16            | 4  | 0.07±0.01 | +           | +      |
| C. sinuosa      | Summer/11    | 9        | 22            | +  | 0.24±0.02 | +           | +      |
| D. ciliolata     | Summer/11    | 7        | 16            | 11 | 0.14±0.02 | F = 126.3; p< 0.001 | 13.9   |
| D. crispata     | Summer/11    | 7        | 16            | 19 | 0.14±0.04 | F = 646.9; p< 0.001 | 25.7   |
| D. delicatula   | Spring/09    | 2        | 8             | 8  | 0.14±0.01 | F = 31.8; p< 0.001 | 7.7    |
| D. delicatula   | Summer/11    | 3        | 12            | 10 | 0.08±0.01 | F = 6.8; p< 0.001 | 19.1   |
| D. delicatula   | Summer/11    | 7        | 16            | +  | 0.13±0.01 | +           | +      |
| D. delicatula   | Summer/11    | 8        | 20            | +  | 0.12±0.02 | +           | +      |
| D. dichotoma    | Summer/11    | 7        | 6             | +  | 0.11±0.01 | +           | +      |
| D. mertensii    | Spring/09    | 2        | 8             | 7  | 0.19±0.01 | F = 15.7; p< 0.001 | 5.2    |
| D. mertensii    | Summer/11    | 8        | 20            | +  | 0.10±0.01 | +           | +      |
| D. mertensii    | Summer/11    | 9        | 22            | 10 | 0.18±0.03 | F = 73.9; p< 0.001 | 15.4   |
| D. pfaffii      | Summer/11    | 3        | 12            | 10 | 0.10±0.02 | F = 34.1; p< 0.001 | 16.6   |
| D. polypodioides| Summer/11    | 8        | 20            | +  | 0.22±0.01 | +           | +      |
| L. variegata    | Spring/09    | 2        | 8             | 10 | 0.91±0.22 | F = 366.0; p< 0.001 | 24.1   |
| L. variegata    | Summer/11    | 3        | 2             | +  | 0.13±0.00 | +           | +      |
| L. variegata    | Summer/11    | 7        | 16            | 10 | 0.81±0.53 | F = 3765.0; p< 0.001 | 65.3   |
| P. gymnospora   | Autumn/11    | 1        | 6             | +  | 0.40±0.02 | +           | +      |
| P. gymnospora   | Spring/09    | 2        | 8             | 10 | 0.07±0.01 | F = 58.1; p< 0.001 | 13.1   |
| P. gymnospora   | Summer/11    | 4        | 14            | +  | 0.19±0.01 | +           | +      |
| P. gymnospora   | Summer/11    | 5        | 14            | +  | 0.26±0.02 | +           | +      |
| P. gymnospora   | Summer/11    | 6        | 14            | +  | 0.05±0.00 | +           | +      |
| Species          | Season/Year | Site | Date | Biomass | F (df)   | p-value | % Multiplied |
|------------------|-------------|------|------|---------|----------|---------|--------------|
| P. gymnospora    | Summer/11   | 7    | 16   | 7       | 0.13±0.05 | $F = 127.3; p < 0.001$ | 42.7 |
| P. gymnospora    | Summer/11   | 8    | 20   | +       | 0.09±0.02 | +       | +            |
| P. gymnospora    | Summer/11   | 9    | 22   | +       | 0.22±0.09 | +       | +            |
| P. gymnospora    | Autumn/10   | 10   | 7    | 8       | 0.58±0.30 | $F = 802.3; p < 0.001$ | 51.8 |
| S. filipendula   | Summer/11   | 3    | 12   | 9       | 0.09±0.00 | $F = 48.9; p < 0.001$ | 7.6 |
| S. filipendula   | Summer/11   | 4    | 14   | 10      | 0.38±0.10 | $F = 1166.8; p < 0.001$ | 25.6 |
| S. ramifolium    | Summer/11   | 8    | 20   | 7       | 0.17±0.06 | $F = 166.5; p < 0.001$ | 36.5 |
| S. Schroederi    | Summer/11   | 8    | 20   | 10      | 4.30±0.78 | $F = 180.1; p < 0.001$ | 18.1 |
| S. stenophyllum  | Autumn/10   | 10   | 27   | 7       | 0.45±0.19 | $F = 109.9; p < 0.001$ | 42.0 |
| S. vulgare       | Spring/09   | 2    | 8    | 15      | 0.13±0.01 | $F = 85.8; p < 0.001$ | 9.6 |
| S. vulgare       | Summer/11   | 4    | 14   | 6       | 0.14±0.04 | $F = 4335.0; p < 0.001$ | 26.3 |
| S. vulgare       | Summer/11   | 5    | 14   | 12      | 0.73±0.15 | $F = 90.5; p < 0.001$ | 20.7 |
| S. vulgare       | Summer/11   | 6    | 14   | 10      | 0.20±0.11 | $F = 1428.1; p < 0.001$ | 53.9 |
| S. vulgare       | Summer/11   | 7    | 16   | 10      | 0.10±0.02 | $F = 89.9; p < 0.001$ | 18.1 |
| S. vulgare       | Summer/11   | 9    | 22   | 5       | 1.10±0.31 | $F = 212.8; p < 0.001$ | 30.9 |
| S. Zonale        | Summer/12   | 9    | 22   | 32      | 1.72±0.49 | $F = 37.2; p < 0.001$ | 28.3 |

1. Tibau do Sul; 2. Recife; 3. Salvador; 4. Uruçuca; 5. Ilhéus (Morro de Pernambuco); 6. Ilhéus (Back Door); 7. Porto Seguro; 8. Guarapari; 9. Armação dos Búzios; 10. Florianópolis.

* Insufficient biomass for individual analysis.