FEEDING-DETERRENT PROPERTIES OF DITERPENES OF *Dictyota mertensii* (PHAEOPHYCEAE, DICTYOTALES)*

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ABSTRACT

Crude extracts of the brown seaweed *Dictyota mertensii* (Martius) Kützing collected at two distant and different places on the Brazilian coast, Búzios (Rio de Janeiro) and Fernando de Noronha (PE), were evaluated for defensive chemistry against the crab *Pachygrapsus transversus*, and the sea urchin *Lytechinus variegatus.* The extract from Búzios specimens of *D. mertensii* significantly inhibited the consumption by both *P. transversus* and *L. varigetaus*. Fractionation of the extracts of specimens of *D. mertensii* from Búzios and F. de Noronha followed by complementary assays revealed one active fraction from each location, which contained distinct defensive secondary metabolites. In each active fractions prenylated guaiane diterpenes were the major compounds. Dictyol H and epoxypachydictyol A were the most abundant compounds in Búzios and F. de Noronha, respectively, followed by minor components. Our results show a differential production of secondary metabolites in the two distant and different populations of *D. mertensii* along the Brazilian coast. This suggests that defensive chemicals from this seaweed are not qualitatively absolute characteristics of the species, but may represent an ecological specialization to successfully prevent herbivory.

RESUMO

Os espécimes da macroalga parda *Dictyota mertensii* coletados em dois diferentes locais no litoral brasileiro e distantes entre si, Búzios (Rio de Janeiro) e Fernando de Noronha (PE), foram avaliados quanto à atuação de seus extratos como defesa química contra o caranguejo *Pachygrapsus transversus*, e o ouriço do mar *Lytechinus variegatus.* O extrato dos espécimes de *D. mertensii* de Búzios inibiu o consumo por ambos *P. transversus* e *L. variegatus*. O fracionamento dos extratos dos espécimes de *D. mertensii* de Búzios e F. de Noronha avaliados em ensaios complementares revelaram uma fração ativa contendo distintos metabolitos secundários defensivos. Diterpenos do tipo guaiano prenilado foram as substâncias majoritárias em ambas as frações, mas dictyol H e epoxipachydictyol A foram os mais abundantes em Búzios e F. de Noronha, respectivamente, seguidos por componentes minoritários. Nossos resultados mostram uma produção diferencial de metabolitos secundários em duas populações distintas e distantes de *D. mertensii* no litoral brasileiro e sugerem que as defesas químicas desta macroalga marinha não são uma característica qualitativa absoluta desta espécie e pode representar uma especialização ecológica eficaz para prevenir a herbivoria.

Descriptors: Diterpenes, *Dictyota mertensii*, Feeding, Chemical defense variation.
Descritores: Diterpenos, *Dictyota mertensii*, Herbivoria, Variação de defesa química.

INTRODUCTION

Many seaweed secondary metabolites which are qualitatively or quantitatively variable appear to be important in mediating interactions with herbivores and other marine micro- and macro- organisms (Paul *et al.,* 2001; 2006). Then, the knowledge of both the variation of secondary metabolites of marine organisms is a key element for studies in chemical ecology, particularly if put into an ecological and evolutionary context (Schmitt *et al*., 1995; De Nys *et al*., 1995; 1998; Hay *et al*., 1998)

In general, the intraspecific patterns of qualitative or quantitative variation in chemical defenses are largely undocumented in marine organisms (but see Van Alstyne *et al*., 1999), and thus underappreciated (Hay, 1996). However, due to the known and broad ecological roles of seaweed secondary metabolites (Paul *et al*., 2001), these variations may be of significant importance and/or repercussion for populations and community structure.

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In general, secondary metabolites vary in seaweeds, but it is not known whether these variations are genetically or environmentally controlled (result of long evolutionary or actual ecological pressures, respectively). For example, lipophylic compounds such as terpenoids in seaweeds occur in lower concentrations, ranging from 0.2% to 2.0% of algal dry mass (DM) (Paul & Fenical, 1986; Hay & Fenical, 1988), while polyphenolic compounds in brown seaweeds can occur at concentrations as high as 15.0% of algal DM (Ragan & Glombitza, 1986). Their concentrations, however, can not be predicted by latitude alone (Targett *et al*., 1992), as previously thought. The variation in secondary metabolites may occur among individuals within a population (Paul & Van Alstyne, 1988a;b; Puglisi & Paul, 1997; Matlock *et al*., 1999) or among populations of the same species growing in different habitats (Paul & Fenical, 1986; 1987; Paul *et al*., 1987; Paul & Van Alstyne, 1988a). For example, populations of the green seaweed *Halimeda* from habitats with high herbivory contain higher amounts of the potent defense terpenoid halimedatrial than populations from areas with low herbivory (Paul & Van Alstyne, 1988a).

Different secondary metabolites may also be found in the same species of seaweed growing in different habitats (Hay & Fenical, 1988; Pereira *et al*., 2000). For example, shallow and deep-water populations of the brown seaweed *Stypopodium zonale* (Lamouroux) Papenfuss produce different secondary metabolites (Gerwick *et al*., 1985), and the red alga *Portieria hornemannii* (Lyngbye) P. Silva varies in its composition of halogenated monoterpenes among different collection sites in the tropical Ocean (Gunatilaka *et al*., 1999). Accordingly, the brown seaweed *Dictyota menstrualis* (Hoyt) Schnetter, Hörnig et Weber-Peukert differs in its major metabolite composition from the northwestern Atlantic (North Carolina; Hay, 1996) to the southwestern Atlantic Ocean (Rio de Janeiro; Pereira *et al*., 2000). In another latitudinal scale, natural concentrations of the extracts from *S. zonale* from Búzios and Fernando de Noronha significantly deterred feeding by the amphipod *P. transversus* and the sea-urchin *L. variegatus*, although the former was more effective as a defense than the latter (Pereira *et al*., 2004). Corroborating these results, the major metabolites atomaric acid, found in individuals from Búzios, and stypoldione, from Fernando de Noronha specimens, also inhibited herbivory, but atomaric acid was more effective as a defense against *L. variegatus* and *P. transversus* than stypoldione, suggesting the existence of a geographic variation in secondary metabolites that has not been previously identified. In order to ascertain the nature of chemical defenses among Brazilian populations of *Dictyota mertensii*, the crude organic extracts from two populations of this seaweed were evaluated as defense against herbivores to specifically answer the following questions: 1) Is there variation in *D. mertensii* secondary metabolite quality (crude extracts and fractions) from north to south along the Brazilian coastal zone? 2) Are there geographic implications for the distribution of the alga?

Material and Methods

Organisms and Study Sites

Specimens of the brown alga *Dictyota mertensii* were colleted at Praia Rasa, located at Armação de Búzios, State of Rio de Janeiro, southeast coast, Brazil and at Fernando de Noronha Archipelago, northeast coast, Brazil.

Praia Rasa is located between Armação de Búzios and Cabo Frio (22°44´10 S and 42 °57´50 W). It has a rocky shore with a gentle slope about 20 m wide and it is considered to have the most diverse algal flora of the state (Pereira *et al*., 1994; Yoneshigue, 1985). In the infralittoral fringe, a dense cover of *Sargassum* spp. can be observed during the lowest tides forming a continuous belt about 5 m wide along the shore where *Sargassum furcatum* is one of the most abundant algal species (Yoneshigue, 1985; Pereira & Yoneshigue, 1999).

The Archipelago of Fernando de Noronha consists of one large island and 19 small, adjacent islets, totaling ca. 26 km^2 (Maida & Ferreira, 1997). According to Eston *et al*. (1986), Resurreta channel has the highest algal diversity among the sites they studied. From 2 m to 9 m depth, the local benthic community is dominated by the brown seaweeds *Dictyopteris* spp., *S. zonale*, *Sargassum* spp. and *Dictyota* spp. Other common seaweeds include *Caulerpa verticillata* J. Agardh, *Amphiroa* spp., and *Lobophora variegata* Lamouroux. From 20 to 30 m depth, the zone of *Montastrea cavernosa* (Linnaeus) (Anthozoa) colonies growing as large pinnacles can be found. A total of ninety-five species of fishes are known from the Fernando de Noronha region (Maida & Ferreira, 1997), including endemic species such as *Stegastes rocacensis* (Emery) and *Thalassoma noronhanum* (Boulenger).

Both herbivores used in this study, the rock crab *Pachygrapsus transversus* (Gibbes) and the sea urchin *Lytechinus variegatus* (Lamarck) are very common organisms found all along the Brazilian coast and were collected at Boa Viagem Island and Itaipu Beach, respectively, in Niterói city, Rio de Janeiro State.

Extraction procedures and chemical analysis

The powdered dry alga (specimens of *D. mertensii*) was exhaustively extracted with dichloromethane (CH_2CL_2) and the solvent was further evaporated under reduced pressure. The residue of the crude extract was subjected to silica gel column chromatography eluting with increasing polarity solvents to obtain fractions for testing (feeding deterrence assays). The active fractions were submitted to further silica gel column chromatography to purify the active component and to test it against herbivores. Thin Layer Chromatography (TLC) analyses were used to compare the composition of the crude extracts from specimens of *D. mertensii* collected at Búzios and that from Fernando de Noronha. The presence and characteristic of the natural products were verified by ^{1}H-NMR (300MHz, $CDCl_3$) analyses and comparison with spectral data previously published in the literature.

Feeding deterrence assays

Feeding deterrence of *D. mertensii* extracts was verified using natural concentrations of algal extracts in an artificial food prepared according to Hay *et al*. (1994). Artificial foods (treatment- with extract, and control – without extract) were added to a small square mesh (10 mm x 10 mm squares) and simultaneously offered to herbivores. All assays were run in small cups, each containing 1 individual of the crab *P. transversus* (or *Lytechinus variegatus*, in sea-urchin assays) together with control and treatment foods. The effect of crude extract on herbivory was determined by comparison of the number of squares consumed in both treatment and control foods. The Wilcoxon statistic test (Zar, 1998) was used to verify significant difference ($p < 0.05$) of consumption between treatment and control treatments.

RESULTS AND DISCUSSION

Crude Extract

The crude extract residue of *D. mertensii* from Praia Rasa significantly inhibited ($p < 0.05$) the consumption by *P. transversus*, when compared to the respective control (Fig. 1). In the same way, the control consumption by the sea urchin *L. variegatus* was significantly larger ($p < 0.05$) than the treatment consumption (Fig. 2). Therefore, this crude extract of *D. mertensii* exhibited significant chemical defensive properties against feeding by both herbivores. Due to these successful results with both herbivores the subsequent assays to verify the active compound present in the crude extract, used only the crab *P. transversus*.

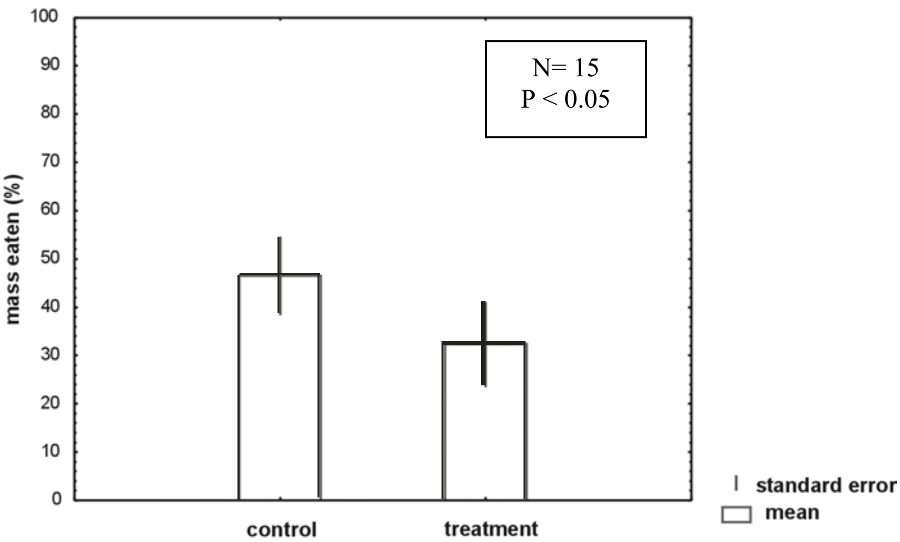

Fig. 1. Feeding by *P. transversus* on paired control and treatment containing crude extract of *D. mertensii* from Praia Rasa (Búzios, RJ). Vertical bars = standard deviations. P= t test value.

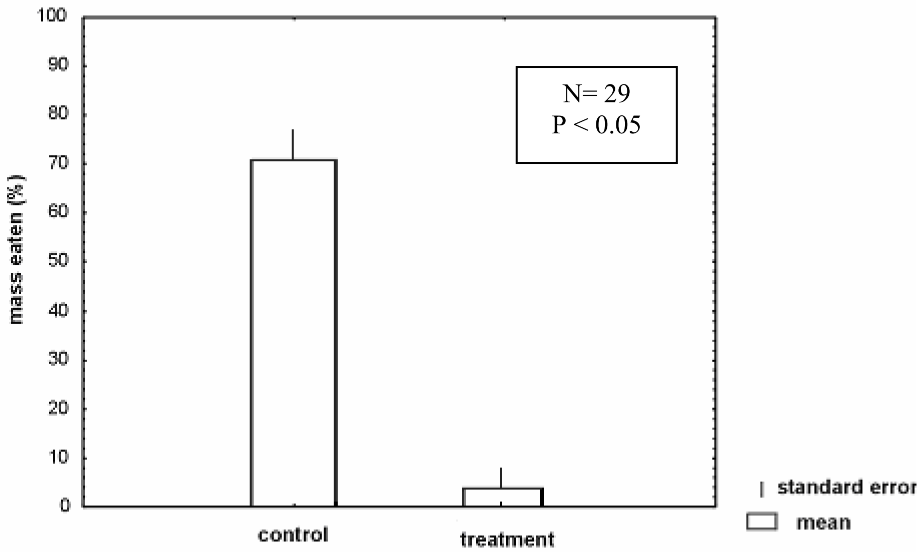

Fig. 2. Feeding by *L. variegatus* on paired control and treatment containing crude extract of *D. mertensii* from Praia Rasa (Búzios, RJ). Legends as in the Fig. 1.

Fractions

All fractions obtained from the extracts of *D. mertensii* from Búzios and Fernando de Noronha were tested against the crab, but only one fraction from each region was significantly active as defense against *P. transversus*.

From an extract of the specimens of *D. mertensii* from Búzios, the active fraction as defense against *P. transversus* (Fig. 3) possessed the known prenylated guaiane diterpene so called dictyol H (Fig. 4) as major secondary metabolites, and several other minor compounds. To identify this molecule in the crude extract, literature data from prenylated guaianes especially that isolated from Australian *Dictyota volubilis* were used (König *et al.*, 1993; Wright *et al.*, 1993).

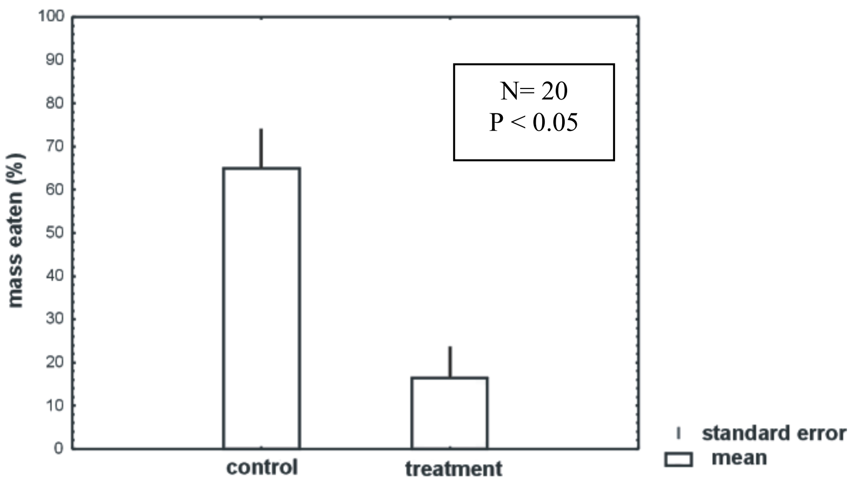

Fig. 3. Feeding by *P. transversus* on paired control and treatment containing one active fraction of the crude extract of *D. mertensii* from Praia Rasa (Búzios, RJ). (n= 13; p < 0.05). Legends as in the Fig. 1.

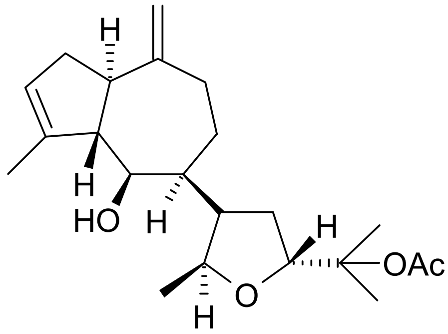

Fig. 4. Structure of the major diterpene found in the active fraction obtained of the crude extract of *D. mertensii* from Búzios, RJ.

One fraction obtained from the crude extract of *D. mertensii* collected at Fernando de Noronha also exhibited freeding deterrence against *P. transverses* (Fig. 5) but was accompanied by high mortality during the experiment (a total of 16 individuals). Epoxypachydictyol A (Fig. 6), also a prenylated guaiane diterpene, was the major compound in the active fraction, followed by several other minor secondary metabolites.

TLC comparisons between sites showed a general pattern for *D. mertensii* with particular geographical characteristics. Although this demonstrates geographical variability for a species, these results are not surprising. Other studies have verified that coral reefs algae or algae from other areas submitted to high feeding pressures, exhibit

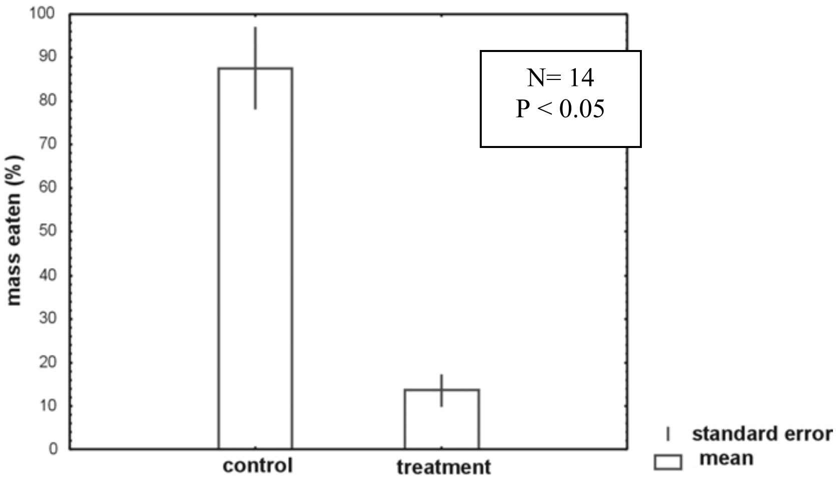

Fig. 5. Feeding by *P. transversus* on paired control and treatment containing one active fraction of the crude extract of *D. mertensii* from Fernando de Noronha, PE. ($n = 4$, $p < 0.05$). Legends as in the Fig. 1.

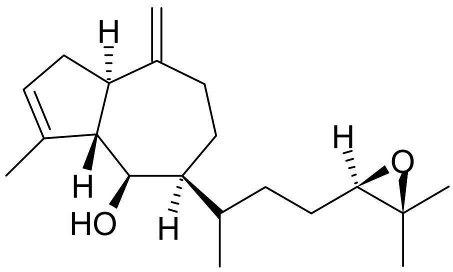

Fig. 6. Structure of the major diterpene found in the active fraction obtained of the crude extract of *D. mertensii* from Fernando de Noronha, PE.

larger concentrations of crude extract or even of active substances against herbivores (Hay & Steinberg, 1992), than others in less pressured environments. In addition, variations in types and concentrations of seaweed secondary metabolites occur at a number of levels, including within and among individuals in a population, with the age of the individuals, and among populations growing in different habitats or geographic regions (Van Alstyne *et al*., 2001).

Finally it should be pointed out that knowledge about the geographical variability in secondary metabolites is important to promote the environmental protections of species diversity, rather than merely for the species with the largest population number.

Our results demonstrated that the different defensive chemicals in two distinct and distant populations of *D. mertensii* are not qualitatively or quantitatively absolute or invariant characteristics of this species, but may represent an ecological specialization to successfully prevent herbivory in a locality.

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REFERENCES

DE NYS, R. *et al.* Broad spectrum effects of secondary metabolites from the red alga *Delisea pulchra* in antifouling assays. **Biofouling**, v. 8, p. 259–271, 1995.

DE NYS, R.; DWORJANYN, A.; STEINBERG, P. D. A new method for determining surface concentrations of natural products on seaweeds. **Mar. Ecol. Prog. Ser.**, v. 162, p. 79–87, 1998.

ESTON, V. R. *et al.* Vertical distribution of benthic marine organisms on rocky coast of the Fernando de Noronha Archipelago (Brazil). **Bolm Inst. oceanogr.**, S. Paulo, v. 34:37–53, 1986.

GERWICK, W. H.; FENICAL, W.; NORRIS, J. N. Chemical v ariation in the tropical seaweed *Stypopodium zonale* (Dictyotaceae). **Phytochemistry**, v. 24, p. 1279–1283, 1985.

GUNATILAKA, A. A. L. *et al.* Apakaochtodenes A and B: two tetrahalogenated monoterpenes from the red marine alga *Portieria hornemannii*. **J. Nat. Prod.**, v. 62, p. 1376–1378, 1999.

HAY, M. E. Marine chemical ecology: what's known and what's next? J. expl. **Biol. Ecol.**, v. 200, p. 103–134, 1996.

HAY, M. E.; FENICAL, W. Marine plant-herbivore interactions: the ecology of chemical defense. **Ann. Rev. Ecol. Syst.**, v. 19, p. 111–145, 1988.

HAY, M. E.; STEINBERG, P. D. The chemical ecology of plant herbivore interactions in marine versus terrestrial comunities. In: ROSENTHAL, G. A.; BERENBAUM, M. R. (Ed.). **Herbivores** - their interactions with secondary plant metabolites. New York: Academic Press, 1992. p. 371-413.

HAY, M. E.; KAPPEL, Q. E.; FENICAL, W. Synergisms in plants defenses against herbivores: intercations of chemistry, calcification, and plant quality. **Ecology**, v. 75, p. 1714-1726, 1994.

HAY, M. E. *et al*. Bioassays with marine and freshwater macroorganisms. In: HAYNES, K. F.; MILLAR, J. G. (Ed.). **Methods in chemical ecology**, v.2. New York: Chapman and Hall, 1998. p.39-141.

KÖNIG, G. M. *et al*. Four new hydroazulenoid diterpenes from the tropical marine brown alga *Dictyota volubilis.* **Planta Med.**, v. 59, p. 174-178, 1993.

MAIDA, M.; FERREIRA, B. P. Coral reefs of Brazil: an overview. In: INTERNATIONAL CORAL REEF SYMPOSIUM, 8., 1996, Panama. **Proceedings**… Balboa, Panama: Smithsonian Tropical Research Institute, 1997. v. 1, p. 263–274.

MATLOCK, D. B.; GINSBURG, D. W.; PAUL, V. J. Spatial variability in secondary metabolite production by the tropical red alga *Portieria hornemannii*. **Hydrobiologia**, v. 399, p. 267–273, 1999.

PAUL, V. J.; FENICAL, W. Chemical defense in tropical green algae, order Caulerpales. **Mar. Ecol. Prog. Ser.**, v. 34, p. 157–169, 1986.

PAUL, V. J.; FENICAL, W. Natural products chemistry and chemical defense in tropical marine algae of the Phylum Chlorophyta. In: SCHEUER, P.J. (Ed.). **Bioorganic marine chemistry**. v. I. Berlin: I. Springer-Verlag, 1987. p. 1–37.

PAUL, V. J.; VAN ALSTYNE, K. L. Antiherbivore defenses in *Halimeda*. In: INTERNATIONAL CORAL REEF SYMPOSIUM, 6., 1988, Townsville. **Proceedings**… [Townsville, Qld.?]: Organizing Committee of the Sixth International Coral Reef Symposium, 1988a. v. 3, p. 133–138.

PAUL, V. J.; VAN ALSTYNE, K. L. Chemical defense and chemical variation in some tropical Pacific species of *Halimeda* (Chlorophyta, Halimedaceae). **Coral Reefs**, v. 6, p. 263–270, 1988b.

PAUL, V. J. *et al.* Evidence for chemical defense in the tropical green alga *Caulerpa ashmeadii* (Caulerpaceae: Chlorophyta): isolation of new bioactive sesquiterpenoids. **J. chem. Ecol.**, v. 13, p. 1171–1185, 1987.

PAUL, V. J.; CRUZ-RIVERA, E.; THACKER, R. W. Chemical mediation of macroalgal herbivore interactions: ecological and evolutionary perspectives. In: MCCLINTOCK, J. B.; BAKER, B. J. (Ed.). **Marine chemical ecology**. Baton rouge, Fla: CRC, 2001. p. 227–265.

PAUL, V. J.; PUGLISI, M. P.; RITSON-WILLIAMS, R. Marine chemical ecology. **Nat. Prod. Rep.**, v. 23, p. 153–180, 2006.

PEREIRA, R. C.; YONESHIGUE-VALENTIN, Y. The role of polyphenols from the tropical brown alga *Sargassum furcatum* on the feeding by amphipod herbivores. **Bot. Mar.**, v. 42, p. 441-448, 1999.

PEREIRA, R. C.; CAVALCANTI, D. N.; TEIXEIRA, V. L. Effects of secondary metabolites from the tropical Brazilian brown alga *Dictyota menstrualis* on the amphipod *Parhyale hawaiensis*. **Mar. Ecol. Prog. Ser.**, v. 205, p. 95–100, 2000.

PEREIRA, R. C. *et al.* Variation in chemical defenses against herbivory in southwestern Atlantic *Stypopodium zonale* (Phaeophyta). **Bot. Mar.**, v. 47, p. 202–208, 2004.

PEREIRA, R. C.; TEIXEIRA, V. L.; KELECOM, A. Chemical defenses against herbivores in marine algae. I. The brown alga *Dictyota dichotoma* (Hudson) Lamouroux from Brazil. **An. Acad. bras. Ci.**, v. 66, p. 229-235, 1994.

PUGLISI, M. P.; PAUL, V. J. Intraspecific variation in secondary metabolite production in the red alga *Portieria hornemannii*: monoterpene concentrations are not influenced by nitrogen and phosphorus enrichment. **Mar. Biol.**, v. 128, p. 161–170, 1997.

RAGAN, M. A.; GLOMBITZA, K. W. Phlorotannins: brown algal polyphenols. **Prog. Phycol. Res.**, v. 4, p. 129–241, 1986.

SCHMITT, T. M.; HAY, M. E.; LINDQUIST, N. Constraints on chemically mediated coevolution: Multiple functions for seaweed secondary metabolites. **Ecology**, v. 76, p. 107–123, 1995.

Targett, N. M. *et al*. Biogeographic comparisons of marine algal polyphenolics: evidence against a latitudinal trend. **Oecologia**, v. 89, p. 464–470, 1992.

VAN ALSTYNE, K. L. *et al*. Geographic variation in polyphenolic levels of Northeastern Pacific kelps and rockweeds. **Mar. Biol.**, v. 133, p. 371–379, 1999.

VAN ALSTYNE, K. L.; DETHIER, M. N.; DUGGINS, D. O.. Spatial patterns in macroalgal chemical defenses. In: MCCLINTOCK, J. B.; BAKER, B. J. (Ed.). **Marine chemical ecology**. Boca Raton, Fla: CRC, 2001. p. 301-324.

WRIGHT, A. D.; KÖNIG, G. M.; STICHER, O. New and highly oxidised hydroazulenoid diterpenes from the tropical marine brown alga *Dictyota volubilis.* **Tetrahedron**, v. 49, p. 571-580, 1993.

ZAR, J. H. **Biostatistical analysis**. 4. ed. Englewood Cliffs, NJ: Prentice-Hall, 1998. p. 929.

Sources of Unpublhished Material

YONESHIGUE, Y. **Taxonomie et ecologie des algues marines dans la région de Cabo Frio (Rio de Janeiro, Brésil)**. 1985. 466 f. Thesis (PhD. Sci.) - Fac. Sci. Luminy, Univ. Aix-Marseille II.

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