EXPERIMENTAL INVESTIGATION OF CHANGE IN CHEMICAL COMPOUNDS OF THE INVASIVE CORAL *TUBASTRAEA* SPP. DUE TO PROXIMITY OF COMPETITORS

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INTRODUÇÃO

Competition for space is usually described in sessile organisms when one species may eliminate another by competitive exclusion. Benthic organisms are subjected to intense rates of predation and competition and the presence of secondary metabolites, which may defend individuals or species, appear to be common, and ecologically important (McClintok & Baker, 2001). Cnidarians are considered to be groups rich in chemicals, producing an array of chemical metabolites many of which are assumed have a defensive role. Chemically mediated interactions in soft corals have been well studied (Coll, 1992) and many of these produced compounds are known to possess negative action against predators (Fleury *et al.*, 008) or competitors (Lages *et al.*, 006). In the same way, some experiments focusing on scleractinian corals have also showed bioactive compounds (Rashid *et al.*, 995) some of which may function as allelochemicals (Koh & Sweatman, 2000).

The ahermatypic scleractinian corals *Tubastraea coccinea* Lesson, 1829 and *T.tagusensis* Wells, 1982 have recently been detected in Brazilian waters and they are well - established on the rocky shores at Ilha Grande Bay, southeast Brazil (Paula & Creed, 2004). Marine alien species are modifying many areas around world and it brings negative and irreversible consequences for all community including economic impact (Occhipinti - Ambrogi, 2007). The competition for space can affect the species distribution and community organization and the presence of chemical metabolites in tissues of alien species may to aid them establishes and expands more easily in the new places (Pereira, 2004). However, no studies in brazilian waters have shown variability in secondary metabolites meditates competition for space between organisms.

OBJETIVOS

The focus of the present study was to experimentally detect variation in chemical compounds which potentially mediate competition produced by the alien species *T. coccinea* and *T. tagusensis* when in close proximity to native species *Mussismilia hispida* (Verrill, 1901) and *Desmapsamma anchorata* (Carter, 1882) at Ilha Grande Bay, RJ, Brazil.

MATERIAL E MÉTODOS

Study area and Experiment

The experiments were carried out at Ilha dos Macacos station, Ilha Grande Bay, located in the south of Rio de Janeiro State, Brazil (23º 04.713'S 44º 13.479'W). The study site was a tropical subtidal rocky reef situated in the Canal Central of the bay. Two commonly occurring native species were chosen for the competitive interaction experiment. *M. hispida* is the most abundant scleractinian hermatipic coral and it is endemic to Brazil and was chosen because previous studies suggested that this coral is negatively affected by the presence of *Tubastraea* spp (Creed, 2006; de Paula, 2007). In contrast, the sponge *D. anchorata* which is also an important space occupying organism at this site was selected because it seemed not to be affected by the presence of the exotic corals (de Paula, 2007). Fleshy colonies and competitors were collected and the experiments run in April, 2007. For the experiment we used five different treatments (n=8 replicates) for each of the two *Tubastraea* species: 1) *Tubastraea* alone (control); 2) *Tubastraea* and *M. hispida*; 3) *Tubastraea* and *D. anchorata*; 4) *Tubastraea* and *M. hispida* mimic; 5) *Tubastraea* and *D. anchorata* mimic. We transplanted the pairs of organisms/mimics 2 cm apart onto ceramic tile (15 x 15 cm) using Tubolit epoxy putty to attach the corals and coral mimics and elastic lines for the sponge and its mimic. The mimics of *D. anchorata* were prepared using flexible rubber sheets wrapped in synthetic sponge and mimics of *M. hispida* were prepared in concrete using the bottom of a plastic bottle as a mold. The use of treatments employing mimics was to identify possible effects on production of chemical compounds by *Tubastraea* spp due to physical presence or contact. All treatments were attached in random order with cable ties to concrete blocks at 2 to 4 m depth. The organisms were left to interact for 5weeks. After this time the native organisms were inspected for the presence of necrosis (retraction of living tissue) or growth.

Chemical extraction and analysis

After 5 weeks in the field, the colonies of *Tubastraea* spp were collected and separated in half with the side in proximity to the potential competitor retained and labeled before being immediately frozen. The frozen tissues of Tubastraea were extracted using methanol (MeOH) solvent, and an aliquot of each extracts was fractioned using dichloromethane (DCM) solvent. The identification of the substances by Gas Chromatography Mass Spectrometry (CG/MS) was comparison with literature data, some co - injections with standard and the Wiley 275 Mass Library.

Statistical Analyses

The data from the CG/MS were used quantitatively to generate proportional abundances of each fraction for each replicate of each treatment. Prior to analysis, the data were arcsine transformed. We use a multivariate analysis of variance MANOVA on the global data set in order to detect variation on concentration of chemical substances between the two species (Wilk's Lambda test). MANOVA was also used for each species of coral to detect differences on concentration of chemical compounds between controls against treatments (Wilk's Lambda test). We used one - way ANOVA and Tukey - tests to identify differences between controls and treatments for individual fractions for each species separately to detect differences on concentration of allelophatic compounds between control and treatments. These analyses were carried out using SPSS for Windows. Complementary multivariate analyses were carried out on mean values per treatment to compare species and treatment similarity by Multi - Dimensional Scaling (MDS). Multivariate analyses (Principal Components Analyses –PCA) using the software Unscrambler, version 9.1 were used to visualize the similarity of data through the variation on concentration of allelophatic compounds produced by control comparing to treatments for each species of *Tubastraea*.

RESULTADOS

The GC/MS analysis of DCM fractions for all treatments of *T. coccinea* and *T. tagusensis* detected 28 and 26 substances, respectively. The multivariate analysis of variance showed significant differences in the chemical composition of the two species (p= 0.004; Wilks' Lambda test, MANOVA).The chemical composition at the genus level showed no significant difference between treatments (p= 0.071; Wilks' Lambda test, MANOVA). We also detected significant differences in the overall chemical composition of extracts between treatments for *T. tagusensis*(p= 0.004; Wilks' Lambda test, MANOVA). The concentration of the alkaloid 5 - Bromoindole - 3 - carbaldehyde showed significant variation between control and treatment (p= 0.044; one - way ANOVA) and control and treatment employing *D. anchorata* were responsible for the variation of this alkaloid (p= 0.042; Tukey test). For *T. coccinea* we detected no significant variation in concentration for all chemical compounds between treatments (p= 0.122; Wilks' Lambda test, MANOVA). However, the concentration of the sterol Ergost - 5,24 - dien - 3 β - ol varied significantly between two treatments (p= 0.034; MANOVA, Wilks' Lambda test) and the treatment employing *M. hispida* mimic and the treatment employing *M. hispida* were responsible for strong tendency on variation of this sterol (p= 0.057; Tuckey test). The direct evidence on the action of allelochemical compounds produced by both *Tubastraea* species against one potential competitor was demonstrated in the field. The colonies of *M. hispida* possessed areas of necrosis which summed more than 50% of living colonies size. Therefore, the effects of allelochemical produced by *Tubastraea* were visually detected in the field by competition for space. In contrast the sponge *D. anchorata* overgrew *Tubastraea* colonies in the field. The allelopathic metabolites produced by *Tubastraea* no affected the expansion of sponge. The results for the treatment *T. coccinea* versus *D. anchorata* mimic and *T. coccinea* versus *M. hispida* mimic showed no distinction between treated and control data. The PCA for *T. tagusensis* control versus *M. hispida* showed that treatments fell into two groups. In this treatment, PC1 explained 69% and PC2 21% of the total data variation, summing 90%. For *T. coccinea* control versus *D. anchorata* we found to separate in two groups and PC1 explained 59% and PC2 17% of total variation, summing 76%. The others interactions between *T. coccinea* and *M. hispida* and *T. tagusensis* and *D. anchorata* using PCA no showed good visualization to distinguish control and treatment.

Allelopathy has been studied in greater detail for sessile invertebrates and many manipulative experiments have been used to demonstrate direct inhibition of one species by another (McClintock & Baker 2001). The evidence of allelopathy was seen here because physical contacts between *T. coccinea* and mimics showed no change on concentration of allelopathic compounds produced by coral excluding physic action on production of these substances. Ours results are according to Creed (2006) that also evidenced necrosis on *M. hispida* tissues caused by *Tubastraea* in competition for space. Thus, the evidence of necrosis suggests that two species of *Tubastraea* are competitively dominant and can reduce or exclude the native coral *M. hispida*. The presence of these exotic species in this area is threatening the native benthos and will aid this coral to increase its range and expand and occupy new regions. The genus *Tubastraea* is known to produce compounds used as potentially useful drugs and others with may have an ecological role to play against predators and competitors (Koh and Sweatman, 2000). The brominated indole derivates (alkaloids) found here has reported cytotoxic activity (Rashid *et al.*, 995). The multifunctional effects of many chemical compounds of *Tubastraea* such as those cited above may give this species an advantage in competition for space against competitors in its non - native range. In our study, sterols represented about 40% of all compounds found for both species of *Tubastraea* and these compounds may be also aiding these exotic species of coral to inhibit the growth of other organisms as *M. hispida* around them. Previous studies have focuses on the intraspecific variation in secondary metabolites mainly in seaweeds group; however, this subject has also been reported in some groups of cnidarians (Paul, 1992). In our experiment, we saw that the concentration of Ergost - 5,24 - dien - 3 β - ol varied significantly between treatments for *T. coccinea* and 5 - bromoindole - 3 - carbaldehyde concentration also varied significantly between control and treatment for *T. tagusensis*. Our study is the first to detect variation of chemical compounds for a scleractinian coral which may mediate competitive interactions between this exotic species and the native fauna and contributes to our understanding of the complexity of the competitive interactions between species and the success of *Tubastraea* in colonizing new habitats. The experiment here demonstrated that *Tubastraea* produced an array of potentially allelopathic compounds but it had no effect in hindering the overgrowth of *D. anchorata* onto *Tubastraea*. The significant difference between control and treatment in the production of the alkaloid by Tubastraea did not have an inhibitive effect on *D. anchorata*. This would suggest that the sponge is a stronger competitor for space and it may have potential for hindering the expansion of *Tubastraea*. We found variation in production of allelopathic substances produced by *Tubastraea* due to constant stress suffered by the coral during competition for space. It shows that allocation of resources may be adaptive under conditions of intense predation and/or competition pressure.

CONCLUSÃO

To date no studies focusing on variability in composition and concentration of secondary metabolites due competition for space in tissues of cnidaria have been carried out. Therefore, this study is the first to show that the exotic coral Tubastraea produced variation on concentration and composition of allelopathic compounds against competitors. Investigating this pattern of variation and its consequences on competitors provides some insight into interactions between alien and native competitors. We may conclude that the exotic corals *T. coccinea* and *T. tagusensis* are heavily defended species and threaten native communities through chemical, as well as other, processes.

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