Chemical defense of an exotic coral as invasion strategy

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Abstract

The invasion success of exotic species has been frequently correlated to abiotic and biotic features of the receptor region and to the biological aspects of the invasive organism. There is, however, no information about defensive chemicals found in invasive species as strategy that could promote or facilitate an invasion in a marine environment. We conducted experimental field assays to verify the potential of secondary metabolites of an Indo-Pacific exotic soft coral, Stereonephthya aff. curvata, as a defensive chemical against generalist fish and as an allelopathic agent against the potential local competitor—the gorgonian Phyllogorgia dilatata—in Arraial do Cabo, on the southeastern coast of Brazil. As a result of our experiments, it was confirmed as an efficient chemical defense against fishes by crude coral hexanic extract. In addition to its role as defense against consumers, the field experimental assay also verified that chemicals from this exotic coral had an allelopathic effect causing large necrosis on tissues of the Brazilian endemic gorgonian P. dilatata. Both defensive strategies observed may facilitate the perpetuation and/or expansion and characterize an expressive, invasive facilitator for S. aff. curvata. The obtained results indicate that this exotic coral species may be a real threat to the biological integrity of the Arraial do Cabo Harvest Reserve, Rio de Janeiro. In addition, the study reveals that defensive chemicals can be used to predict the potential invasiveness of introduced species.

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1. Introduction

The significant impact of exotic species in new regions where they had been introduced, affecting native populations, community and ecosystem structure and function, has been widely recognized (Lodge, 1993; Simberloff, 1996). Today these species are seen as an expressive component of immense global changes currently in progress (Vitousek et al., 1996; Bright, 1999). As a result of human influence, coastal marine environments are considered among the most intensely invaded systems (Grosholz, 2002).

The high environmental and economic costs resulting from non-indigenous species introduction has
generated a significant increase in research and publications on prediction, potential impact, or even species distribution (Kolar and Lodge, 2001; Grosholz, 2002).

Generally, the success of an introduced or alien species has been linked to several biotic (Vermeij, 1991; Schoener and Spiller, 1995; Chapin et al., 1998) or even environmental conditions of the receptor region (Golani, 1993; Moyle and Light, 1996). However, some typical characteristics of the invasive organism, such as genetic variability, body size, physiological tolerance, specific reproductive and dispersion strategies and growth rate may also be equally important in promoting an invasion (Elton, 1958; Ehrlich, 1989; Lodge, 1993; Rejmánek and Richardson, 1996; Reichard and Hamilton, 1997; Ceccherelli et al., 2002; Lee, 2002). There are several consistent and statistically significant correlations between the success of an invasion and the characteristics of the invasive species (Kolar and Lodge, 2001).

In addition, several studies have demonstrated a feeding deterrent role for soft coral crude extracts or secondary metabolites. In fact, chemically mediated interactions play an important role in the life history of alcyonacean soft corals (Sammarco and Coll, 1988, 1992). Soft corals produce a diverse range of secondary metabolites, to which ecological roles have been attributed, including defense against predation (Wylie and Paul, 1989; Van Alstyne et al., 1994) and competition for space with scleractinian corals (Sammarco et al., 1983, 1985; Dai, 1990; Fleury et al., 2004).

Defenses against consumers and competitors (allelopathic defenses) widely distributed in the marine environment have been hypothesized as chemical strategies to exotic species colonization, perpetuation and expansion in a new region (Pereira, 2004). However, there is no field experimental evidence that marine exotic species possessing secondary metabolites really facilitate their perpetuation and expansion in a new region. Nevertheless, such an approach might provide additional subsidies to understand the invasion mechanisms of an exotic species, i.e. given appropriate knowledge of an important stage of biological invasion.

Soft corals (Alcyonacea) are most abundant and diverse in tropical habitats, with the Indo-Pacific region containing the greatest diversity in the world (Fabricius and Alderslade, 2001). The soft coral Stereonephthya aff. curvata (according to Ofwegen, pers. com.) is a new and undescribed species originated in the Indo-Pacific region and was discovered in the Arraial do Cabo region about 10 years ago. It was most likely introduced in the region by oil platform fouling (Ferreira et al., 2004). Our study focused specifically on the secondary metabolites as defensive chemicals utilized by the exotic coral in this new habitat. We used field-based assays to answer the following questions: 1) Do crude extracts produced by S. aff. curvata inhibit predation by generalist fish in the field? 2) Do chemicals present on the surface of S. aff. curvata cause any observable physical damage in the endemic gorgonian Phyllogorgia dilatata Esper, 1806?

2. Methods

2.1. Coral sampling

Both coral species, S. aff. curvata and P. dilatata were collected in shallow waters at Arraial do Cabo, located in the state of Rio de Janeiro, on the southeastern coast of Brazil. Arraial do Cabo (23°44′S–42°W, Fig. 1) is a high latitude tropical region possessing a rich reef fauna and flora (Castro et al., 1995; Guimarães and Coutinho, 1996; Ferreira et al., 1998), including tropical and subtropical components. In the study area, the rocky shore environments were covered by a diverse epilithic algal community (Guimarães and Coutinho, 1996), patches of Palythoa caribaeorum (Duchassaing and Michelotti, 1864), colonies of Millepora alcicornis Linnaeus, 1758, and 4 species of hermatypic corals (Castro et al., 1995). Sponges, ascidians, bryozoans, hidrozoans are also important components of the marine benthos in this region.

2.2. Chemical procedures

All colonies of S. aff. curvata were collected during the autumn (May) of 2001 by SCUBA in Saco dos Cardeiros Bay, at a depth of approximately 8 m (Fig. 1). After collection, the colonies were immediately frozen at −25 ºC. The freeze-dried pieces of S. aff. curvata (169.98 g) were fully extracted 3 times con-
secutively, using different polarity solvents: \emph{n}-hexane, CH$_2$Cl$_2$, EtOAc and MeOH. Evaporation of these solvents under reduced pressure yielded 4 corresponding residues (3.57 g, 1.80 g, 0.23 g and 1.74 g), which were tested in feeding assays.

2.3. Defensive field assays

The defensive property of the 4 organic extracts obtained from \emph{S. aff. curvata} were verified by including natural concentrations of these components in an artificial food prepared according to Pawlik and Fenical (1992). These extracts were incorporated in a treated artificial food, at the same volumetric concentration occurring in the living organisms (\emph{n}-hexane: 474.3 mg; CH$_2$Cl$_2$: 188.1 mg; EtOAc: 45.9 mg; MeOH: 294.6 mg, to a final volume of 90 cm$^3$). Carrageenan food strips were prepared combining 3.75 g carrageenan (Sigma C-1013 type 1), 60 ml water and 30 ml commercial tuna fish purée packed in water. The mixture was vigorously stirred to homogenize the lipophilic components of the extract into the carrageenan matrix. The mixture was then poured into specific acrylic molds crossed by lengths of cotton string producing from the ends of the molds. Control strips were prepared without the addition of extract. For each experiment, 30 treated and 30 control food strips (1.0 × 0.6 × 5.0 cm each) were arranged in pairs and attached to 30 ropes. The ropes were randomly anchored in shallow water (2 to 6 m) along the coastal line, and exposed to generalist common tropical fish commonly found at Pedra Vermelha Bay, Arraial do Cabo, RJ (Ferreira et al., 2001). Within 4 to 6 h the ropes were retrieved and the amount of each strip eaten was measured. The Wilcoxon pair sample test was used to analyze the results (one-tailed; Zar, 1996).

2.4. Manipulative field experiments

A set of field experiments were carried out to verify whether the chemical constituents of the exotic
coral *S. aff. curvata* had an allopathic role against the endemic gorgonian *P. dilatata*, including the manipulation of live colonies and corresponding mimics (Fig. 2). The mimics were used to certify that the obtained results would be only due to chemicals and not by physical interactions of both corals when in eventual contact.

Six adult colonies of the coral species, *S. aff. curvata* and *P. dilatata*, were collected on Saco dos Cardeiros and transported to Pedra Vermelha. Pedra Vermelha and Saco dos Cardeiros have very similar environmental conditions (Ferreira et al., 2001).

These corals were labeled and placed on a plastic mesh grid anchored on a substrate approximately 6 m in depth. These specimens were maintained under local environmental conditions for 3 weeks for acclimatization before starting the experiments (as relocated controls, Fig. 2A).

In the general control assays both *S. aff. curvata* (*n* = 6) and *P. dilatata* (*n* = 6) were only observed in Saco dos Cardeiros (non-relocated controls). This was to certify that these colonies would be healthy compared to other colonies used simultaneously in the manipulative experiments or relocated control. Other colonies were also collected for use in the experiments as described.

The allelopathic effect of *S. aff. curvata* on *P. dilatata* was investigated by experiments in which colonies of *S. aff. curvata* were placed in proximity with *P. dilatata* (*n* = 6, Fig. 2B), producing eventual contact (no constant). This procedure facilitated physical contact between these colonies under any current conditions, both anchored in plastic mesh. In this way, this treatment simulated a possibility of contact that could occur under natural conditions. The presence of necrosis on the body of *P. dilatata* was considered as a physical expression of the allelopathic action by *S. aff. curvata* chemicals.

The mimics *S. aff. curvata* were fixed nearby *P. dilatata* in plastic mesh (*n* = 6) to verify the occurrence of eventual necrosis on the body of this gorgonian due to physical contact only (Fig. 2C). At the same time, mimics of *P. dilatata* were fixed nearby *S. aff. curvata* (*n* = 6) to verify the same process. The mimics of *P. dilatata* were prepared with flexible foam rubber sheets attached to a plastic base with “Tubolit”, a special water glue. The mimics of *S. aff. curvata* were prepared with several small and thin pieces of latex hose attached with pumice stone dust, and attached horizontally on the thick and higher piece of the central rubber hose. The central hose was attached to plastic base as mentioned.

The specimens in all treatments and controls (relocated and non-relocated) were monitored weekly for 2 months and the occurrence of tissue necrosis was recorded and photographed. Necrosis was considered to have begun or occurred when the coral tissue changed color or darkened, followed by the exposure of their structural skeleton.

### 3. Results

#### 3.1. Defensive property of the extracts

Of the crude extracts obtained by *n*-hexane, CH$_2$Cl$_2$, EtOAc and MeOH and tested as feeding deterrents on generalist fish, only the *n*-hexanic extract significantly (*p* < 0.05) inhibited feeding by generalist fish (Fig. 3). The conspicuous consumers included fish belonging to the Chaetodontidae, Hae-mulidae, Labridae and Pomacentridae families.
3.2. Manipulative field experiments

All field observations showed that colonies of *S. aff. curvata* and *P. dilatata* remained healthy both in relocated and non-relocated controls during the 3 weeks before the beginning of the assays and during all the experiments. An unexpected contraction of the *S. aff. curvata* colonies was observed. However, this was possibly a natural behavior, as colonies in the Saco dos Cardeiros site were observed as showing the same behavior. Furthermore, species in the Pedra Vermelha site remained healthy living from the beginning to the end of the assays.

From the second week of the experiment, one colony of *P. dilatata* (*n*=1) displayed necrosis due to direct contact with *S. aff. curvata* (Table 1). The remaining colonies (*n*=5) appeared with necrosis signals only after the third week of the experiments. The margins of *P. dilatata* showed brown colors contrasting with natural lilac colors of this gorgonian species (Fig. 4A). Moreover, necrosis in some *P. dilatata* specimens had occurred to such an extent that skeletons were visibly exposed (Fig. 4B). In the experiments where mimics of *S. aff. curvata* were anchored close to *P. dilatata*, these colonies did not display any sign of necrosis. Then, the necrosis observed in *P. dilatata* due to physical contact with live colonies of *S. aff. curvata* was caused specifically by exotic coral chemicals and clearly not by physical contact.

Finally, both mimics and live colonies of *P. dilatata* did not cause any modification on the body of *S. aff. curvata*.

![Fig. 3. Feeding of generalist fish on paired control and treatment containing different extracts of *S. aff. curvata*: (HE) hexanic, (DE) dichloromethane, (EE) ethyl acetate and (ME) methanol. Vertical bars= standard deviations.](image)

![Fig. 4. Phyllogorgia dilatata. Different stages of necrosis appearance after 3 weeks (A) and 8 weeks (B) of assay.](image)

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replicate (n)</th>
<th>Necrosis detection</th>
<th>Necrosis detection on <em>S. aff. curvata</em></th>
<th>Necrosis detection on <em>P. dilatata</em></th>
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<tr>
<td>RCS</td>
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<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
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<td>Yes*</td>
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<tr>
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<td>6</td>
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<td>No</td>
<td>–</td>
</tr>
<tr>
<td>P × Sm</td>
<td>6</td>
<td>No</td>
<td>–</td>
<td>No</td>
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</table>

RCS=Relocated control of *Stereonephthya*; NRCS=Non-relocated control of *Stereonephthya*; RCP=Relocated control of *Phyllogorgia*; NRCP=Non-relocated control of *Phyllogorgia*; S × P=*Stereonephthya* × *Phyllogorgia*; S × Pm=*Stereonephthya* × *Phyllogorgia* mimic; P × Sm=*Phyllogorgia* × *Stereonephthya* mimic.
4. Discussion

Chemical defenses against consumers and competitors (allelopathic defenses) are widely distributed in the marine organisms (McClintock and Baker, 2001) but few works confirmed them experimentally. These chemicals stimulate great interest, as they can be used by marine exotic species during the colonization, expansion and perpetuation processes, important phases of the invasion phenomenon (Pereira, 2004).

Here we show that the exotic coral _S. aff. curvata_, originating from the Indo-Pacific region and introduced in Arraial do Cabo, southeastern Brazil, possesses efficient chemical defense against generalist fish. In addition, when in contact with the endemic gorgonian _P. dilatata_, it causes necrosis by allelopathic action. Some studies in marine environments have noted that secondary metabolites that deter feeding by herbivorous could also serve other functions (Paul, 1992), a multifunctional approach. For example, Schmitt et al. (1995) showed that metabolites that defended the brown seaweed _Dictyota menstrualis_ from herbivorous fish and urchins also served as allelopathic agents that damage the larvae of fouling organisms. However, to our knowledge, this is the first field experimental evidence that an exotic marine species possesses chemicals which can facilitate its perpetuation and expansion in a new region.

The macroalga _Caulerpa taxifolia_ could also have been an example of a chemically defended species that invaded several areas of the northeastern coast of the Mediterranean in Monaco. However, the generalization on the defensive chemicals of _C. taxifolia_ is indirect, due only to the fact that this macroalga is known to possess a sesquiterpene (caulerpenine) also found in other _Caulerpa_ species, making it unpalatable to sea urchins and possibly toxic to fish (Boudour-esque et al., 1996; Amade and Lemée, 1998; Uchimura et al., 1999).

Generally, the survival of some marine organisms in areas submitted to high levels of predation has been previously attributed to a chemically mediated process. In fact, many species of soft corals are known to be chemically well-defended against generalist consumers (Paul, 1992; Sammarco, 1996) and competitors (e.g. Maida et al., 1993; Fleury et al., 2004). It corroborates with our outcomes that the exotic coral _S. aff. curvata_ (Nephtheidae) also produces defensive compounds against fish and potential competitors in a new area of occurrence far from their natural habitat (Indo-Pacific).

Several studies have demonstrated that marine invertebrate secondary metabolites significantly deter consumption by generalist consumers, but often fail to deter, or may even stimulate consumption by specialist consumers. A recent study provided strong evidence that _Chaetodon melanotus_ (Pisces, Chaetodontidae) may feed on alcyonacean corals. Indeed, field observations confirm that corals are an important component of its diet (Alino et al., 1992). Studies with other soft corals species and nudibranches report that specialist organisms may also consume cnidarians and to use toxic compounds against potential predators (Cimino et al., 1982; Coll et al., 1985; Carte and Faulkner, 1986; Pawlik et al., 1988; Wylie and Paul, 1989; Paul et al., 1990; Avila, 1995). It is reasonable to think that the exotic coral _S. aff. curvata_ most likely does not have a specialist predator in the southern Atlantic (Brazil region), because of its recent arrival. Based on thus assumption, its secondary metabolites are taking considerable action against generalist fish, and this species remains and possesses stronger capacity to expand in the Arraial do Cabo region.

In addition to defensive property against consumers, soft coral also produce secondary metabolites which allow them to be extremely competitive with other organisms for space (Coll, 1992; Sammarco and Coll, 1992). Many species of soft corals release chemicals which produce allelopathic effects on surrounding organisms, including hard corals (Sammarco et al., 1985), other soft coral species (La Barre et al., 1986), and seaweed (Coll et al., 1989). For example, the soft coral _Sinularia flexibilis_ which releases the diterpenoid compounds flexibilide and dihydroflexibilide into surrounding water at a concentration of 1 to 5 mg l\(^{-1}\) (Coll et al., 1982), induces necrosis on other soft corals (La Barre et al., 1986). However, these investigations provided observational results about the involvement of chemicals in spatial interference competition—a circumstantial evidence for allelopathy. Here we also showed circumstantial evidence for allelopathy. Although the secondary metabolite involved in this interaction has not been identified or tested in the field, the observed necrosis on the surface and ultimate death of coral polyps of _P. dilatata_ was clearly due to chemicals produced by the exotic coral. Zones of tissue necrosis
(local mortality) on the *P. dilatata* body were observed when the two species were in eventual contact with each other after 2 weeks of assays. In addition, the observed necrosis 2 weeks after the beginning of the experiments supports previous results obtained in similar studies (Sammarco et al., 1985).

Allelopathy is expected to be an important mechanism in the plant invasion process because the lack of co-evolved tolerance of resident vegetation to new chemicals produced by the invader could allow these newly arrived species to dominate natural plant communities (Hierro and Callaway, 2003). Some exotic plants may use this competitive mechanism that are not present in the natural communities that they invade (Callaway and Aschehoug, 2000). In this way, chemical defenses must be either absent in native species or at least less effective than that of the invaders and this is true to our results. The octocoral *P. dilatata* is probably the main competitor of *S. aff. curvata* in Arraial do Cabo rocky shores. It is known to be rich in secondary compounds which, based upon the data available to date, appear to function primarily as anti-predator (Epifânio et al., 1999) or anti-fouling agents (Pereira et al., 2002). However, no signal of allelopathic action of *P. dilatata* against *S. aff. curvata* was observed in the field assays.

Several authors have claimed the importance in understanding what biological characteristics make a species a good invader. In this way, studies have investigated either the success of some invasive species, comparing characteristics in native and invasive species. It might be premature to say the absolute defensive chemistry of the exotic coral against consumers and potential competitors. Nevertheless, it could lead to a predisposition to the invasive success or for the species producing it to become invasive. The present scenario suggest that target exotic coral can be a real threat to the biological integrity of the Arraial do Cabo Marine Harvest Reserve, as well as to other sites along the Brazilian coast.

Finally, although the production of chemical defenses is commonly associated with consumer pressure (herbivory and predation), there are no studies that conclusively demonstrate that herbivores or predators exerted any selective pressure to constitute the abundance and action of current pattern chemical defenses. The introduced soft coral is living quite far from its natural region and it is surviving without known specialist consumers while still producing secondary metabolites. Thus, if invasion is indeed a rapid evolutionary process (see Lee, 2002), chemically defended marine invasive species provide us with an excellent opportunity to search for adaptive responses under the selective pressures of a new environment.

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