CHEMICAL COMPOSITION AND RELEASE *IN SITU* DUE TO INJURY OF THE INVASIVE CORAL *Tubastraea* (CNIDARIA, SCLERACTINIA)*

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ABSTRACT

Defensive chemistry may be used against consumers and competitors by invasive species as a strategy for colonization and perpetuation in a new area. There are relatively few studies of negative chemical interactions between scleratinian corals. This study characterizes the secondary metabolites in the invasive corals *Tubastraea tagusensis* and *T. coccinea* and relates these to an *in situ* experiment using a submersible apparatus with Sep-Paks® cartridges to trap substances released by *T. tagusensis* directly from the sea-water. Colonies of *Tubastraea* spp were collected in Ilha Grande Bay, RJ, extracted with methanol (MeOH), and the extracts washed with hexane, dichloromethane (DCM) and methanol, and analyzed by GC/MS. Methyl stearate and methyl palmitate were the major components of the hexane and hexane:MeOH fractions, while cholesterol was the most abundant in the DCM and DCM:MeOH fractions from *Tubastraea* spp. The organic material retained in Sep-Paks® cartridges was tentatively identified as hydrocarbons. There was a significant difference between treatments and controls for 1-hexadecene, *n*-hexadecane and *n*-eicosane contents. The production of defensive substances by the invasive corals may be a threat to the benthic communities of the region, which include endemic species.

RESUMO

Substâncias químicas de defesa contra consumidores e competidores podem ser usadas por espécies invasoras marinhas como estratégia de colonização e perpetuação em novo ambiente. Entretanto, há poucos estudos experimentais que demonstrem as possíveis interações negativas entre corais escleractínios. Este trabalho tem como objetivo caracterizar os metabólitos secundários dos corais invasores *Tubastraea tagusensis* e *T. coccinea*; avaliar através da técnica de amostragem *in situ* quais são as substâncias de *T. tagusensis* liberadas na água do mar, com o auxílio de aparelho subaquático com colunas Sep-Paks®. Colônias dos corais invasores *Tubastraea* spp foram coletadas na Baía de Ilha Grande, RJ, e extraídas com MeOH. Os extratos foram submetidos à eluições com hexano, DCM e MeOH, e analisados por CG-EM. Estearato de metila e palmitato de metila foram as substâncias majoritárias das frações hexânicas e hexano: DCM, enquanto o colesterol foi a substância mais abundante das frações DCM e DCM:MeOH de *Tubastraea* spp. O material orgânico retido nas colunas Sep-Paks® foi identificado como hidrocarbonetos. Diferenças significativas entre controle e tratamento foram relacionadas a diferentes quantidades de 1-hexadeceno, *n*-hexadecano e *n*-eicosano. A produção de substâncias de defesas em *Tubastraea* spp permite especular sobre a ameaça que estes corais invasores representam para as comunidades bentônicas da Ilha Grande.

Descriptors: Injury, Secondary metabolites, *Tubastraea coccinea*, *Tubastraea tagusensis*, Submersible apparatus.
Descritores: Lesão artificial, Metabólitos secundários, *Tubastraea coccinea*, *Tubastraea tagusensis*, Aparelho submersível.

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INTRODUCTION

Chemical defense against consumers and competitors have been studied in great detail for sessile invertebrates and plants and the detailed mechanisms, in several instances, have been elucidated by manipulative field experiments (HAY; FENICAL, 1988; DE NYS et al., 1991; HAY; STEIN BERG, 1992; PAWLIK, 1993; SCHMITT et al., 1995; THACKER et al., 1998; IRFANULLAH; MOSS, 2005). In the marine environment, the success of defense used by soft corals and gorgonians against consumers and competitors has been attributed to their production of secondary metabolites, many of which show predator deterrence and allelopathic activities (COLL et al., 1982; PAWLIK et al., 1987; DE NYS et al., 1991; VAN ALSTYNE et al., 1994; KELMAN et al., 1999; KOH et al., 2000; LAGES et al., 2006; CHANGYUN et al., 2008).

Despite the fact that hard corals produce a protective external skeleton some experiments have showed that these also produce bioactive compounds (TARRANT et al., 2003; KONTIZA et al., 2006) some of which are deterrent to fish (LAGES et al., 2010) and may function as allelochemicals (KOH; SWEATMAN, 2000).

The compounds involved in allelopathic interactions can act in different concentrations (repellent or toxic) and distances and some of these are soluble or volatile so as to diffuse away from the source (HADFIELD; SCHEUR, 1985; SLATTERY et al., 1997, VAN ALSTYNE et al., 2001). Others may be deposited on the surface of the organism and act by direct contact with potential competitors. The production of secondary metabolites likely requires considerable cost to the organism (COLL, 1992) because the resources allocated to production will not, therefore, be available for other processes such as growth and reproduction (VAN ALSTYNE et al., 2001).

The secondary metabolites used against consumers and competitors may affect the behavior and distribution of organisms in marine systems (LUBCHENCO; GAINES, 1981; ESTES; STEINBERG, 1988; KVITEK et al., 1991; COLL, 1992; STACHOWICZ, 2001). However, compounds responsible for the competitive effects have rarely been isolated and identified (COLL et. al., 1982; SULLIVAN et al. 1983, DE NYS et al. 1991). The use of the submersible sampling apparatus described by Schulte and collaborators (1991) is a method available to collect, *in situ*, trace quantities of organic compounds from seawater. This method provides a simple means of sampling the water around aquatic organisms for bioactive substances thus avoiding the use of large quantities of solvents for extraction (COLL et al., 1982; SCHULTE et al., 1991).

The ahermatypic coral *T. tagusensis* Wells, 1982 is a scleractinian coral non-indigenous to the South Atlantic and was probably introduced into Brazil in the late 1980's (CASTRO; PIRES, 2001) jointly with *T. coccinea* Lesson, 1829. It is well-established on the rocky shores of Ilha Grande Bay, southeastern Brazil (PAULA; CREED, 2004). These species are non-zooxanthellate corals which mainly inhabit overhangs and vertical surfaces (PAULA; CREED, 2005). Studies have demonstrated that this genus produces mainly steroids and alkaloids and that some are toxic to cells and bioactive (FUSETANI et al., 1986; GUELLA et al., 1988; RASHID et al. 1995; IWAGAWA et al., 2008; MEYER et al., 2009). They also may function against competitors (KOH, 1997). Therefore, the chemical study of these alien species is important to understand the behavior in colonization and range expansion. The compounds produced by *Tubastraea* may be aiding these species to establish and persist among the endemic flora and fauna and so change community structure.

This study described the secondary metabolites in the invasive corals *T. tagusensis* and *T. coccinea*, and evaluated, in a field assay, whether *T. tagusensis* releases secondary metabolites in seawater and whether the method applied here may collect, *in situ*, these compounds.

MATERIAL AND METHODS

- Coral material and extraction: *T. coccinea* and *T. tagusensis* were collected at a depth of approximately 3 m from tropical rocky shores at Ilha Itacoatiba (23°4'00"S and 44°15'00"W), located on the southeastern coast of Brazil in the state of Rio de Janeiro. After collection, the colonies were immediately frozen at -25°C. Pieces of different colonies of *T. coccinea* (total weight 665 g) and of *T. tagusensis* (total weight 666 g) were extracted 3 times consecutively using methanol (MeOH) as solvent and an ultrasound machine was used to increase the efficiency of extraction. Evaporation of this solvent under reduced pressure yielded two corresponding brown residues (11.0 g and 9.6g, respectively), that were submitted to an open silica gel column chromatography with hexane, dichloromethane (DCM) and MeOH elution that produced different fractions: hexane (19.1 mg and 6.4 mg), hexane:DCM (1:1) (8.8 mg and 4.5 mg), DCM (17.8 mg and 6.2 mg) and DCM:MeOH (1:1) (278.9 mg and 0.4 mg), respectively. Their secondary metabolites were compared after being analyzed by GC-MS.

- Chemical analysis*:* The gas chromatography mass spectrometry (GC/MS) analysis for substances from MeOH fractions were carried out in a HP Model 5973 with electron impact ionization at 70 eV. A HP5 MS capillary column (30 m × 0.25 mm; coating thickness 0.25 μm / J&W Scientific) was used. Sample volumes of 1μl (DCM solution) were injected in a split mode ratio (1:20) using a manual syringe. Helium was employed as carrier gas. Oven temperature was programmed from 60°C to 290°C at 15°C min^{-1}, where it remained constant for 15 minutes. Injector and detector temperature were kept constant at 280 and 290°C, respectively. The identification of the substances by CG/MS in comparison with literature data (BUDZIKIEWICZ et al. 1964; YAMASHIRO et al., 1999), some co-injections with authentic samples and the Wiley 275 Mass Spectra Library were used.

The GC/MS analysis for compounds trapped into Sep-Paks® columns were carried out using the same capillary column described above. The oven temperature was programmed from 100°C to 290°C at 10°C/min, and it remained constant for 45 minutes. Injector and detector temperatures were also the same as those described above.

- Field experiment: Ten small colonies of *T. tagusensis* were collected in June 2007 on a rocky shore by SCUBA diving at about 2 m depth at Ilha dos Macacos, Ilha Grande Bay, Brazil (23º04.713'S 44º13.479'W). Five living colonies were transplanted to artificial surface (concrete blocks) in the shore along with five skeleton controls that had been previously treated using commercial bleach to remove the tissues and then soaked in running water to remove the bleach. Each colony was individually attached on a concrete block using epoxy putty (Tubolit®). The corals remained *in situ* for 3 weeks at 3 m depth before the beginning of the experiment. Only *T. tagusensis* colonies were used for the experiment because this species is far more abundant in the Ilha Grande Bay (PAULA; CREED, 2005).

The collecting apparatus was constructed in accordance with Schulte et al. (1991). The apparatus consisted of a metal adaptor of four 18 mm long hollow stainless-steel tubes 3 mm in diameter with central opening 1 mm in diameter that were brazed to a basal tube 60 mm long. A single 18 mm long tube was then brazed to the basal tube. This metal frame was duplicated and the two pieces (adaptors) connected by four Sep-Paks® columns (Reversed-phase C_{18}, Waters) (Fig. 1). An inverted funnel was connected to the lower metal adaptor by a silicon hose and the upper metal adaptor was connected to an outlet silicon hose that received compressed air from a SCUBA tank (Fig. 2). Previous studies have suggested that sampling procedures which involve plastic hoses or other similar material should be avoided since the hose has a strongly lipophilic surface capable of adsorbing the allelopathic compounds to be examined, while, at the same time, releasing considerable quantities of plasticizers (FAULKNER et al., 1980). We used an apparatus constructed using silicon hoses to avoid adsorption of compounds by the apparatus.

Before the field experiment, the apparatus were previously tested in a pool by using ink to verify the rate of flow of water moving through Sep-Paks® columns. The substances collecting apparatus were powered by compressed air from a SCUBA tank, whose flow was controlled. A knife was used to cut the colonies inducing an artificial release of chemical compounds in the water following the methods of SCHULTE et al. (1991). The apparatus collected the substances around the species of each replicate during 4-5 hr. The forty Sep-Paks® columns were activated using MeOH solvent before the field experiment. Each colony sampled used four Sep-Paks® columns to capture the substances (see Fig.1) which were pooled into a replicate (n=5 replicates) for subsequent analysis. After each sampling the columns were eluted with 60 mL distilled water, 60 mL MeOH and 60 mL DCM. The MeOH extracts were washed with DCM and the DCM extracts were combined. The differences between control and treatment extracts were detected by GC-MS.

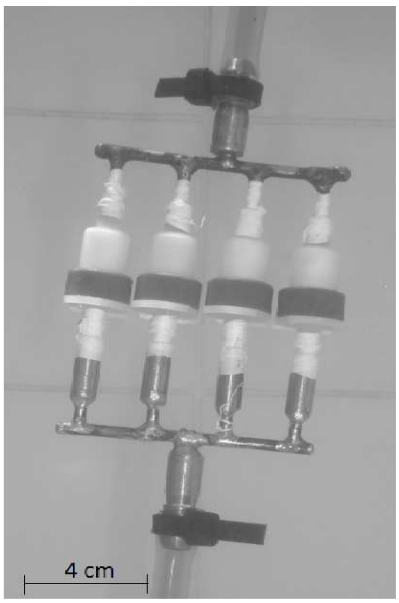

Fig. 1. Metal adaptors with Sep-Paks® in test pool and *in situ* allelopathy experiment.

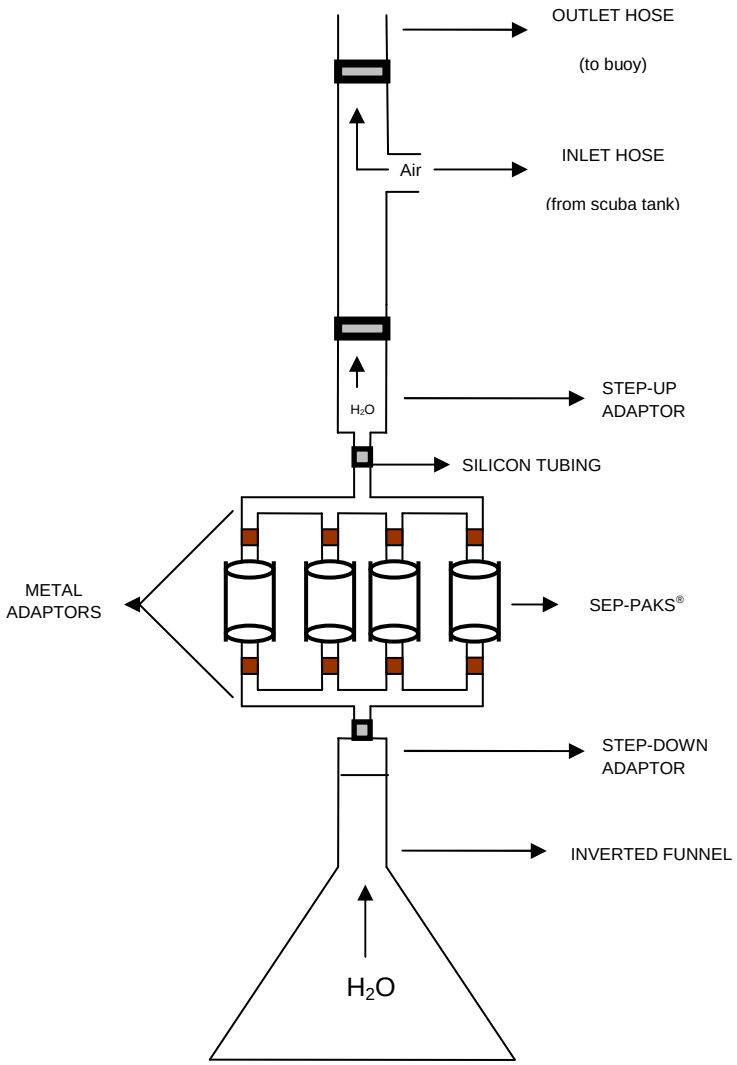

Fig. 2. Schematic representation of the submersible sampling apparatus (adapted from Schulte et al., 1991).

- Statistical Analyses: The table of data (areas of peaks) was transformed to percentage area and arcsine transformed for statistical analysis (QUINN; KEOUGH, 2002). The SPSS package was performed using the non-parametric Mann-Whitney U to test for differences between control and treatments in the detected substances.

RESULTS

Table 1 shows the secondary metabolite contents from different fractions of MeOH extracts of the two invasive species of *Tubastraea* at Ilha Grande Bay, RJ. Thirty-two compounds were tentatively (>90% probability) identified as hydrocarbons, fatty esters and sterols. The hexane and hexane:DCM fractions contained a range of fatty esters (C_{17}-C_{36}) and methyl stearate was found to be the major compound, representing 20.3% and 28.1% (*T. coccinea* and *T. tagusensis*) and 23.6% and 29.2% (*T. coccinea* and *T. tagusensis*, respectively) of the total composition, respectively. Methyl palmitate was also one of the most abundant compounds (17.1% and 19.6%, *T. coccinea* and *T. tagusensis* respectively) in hexane and (18.0% and 21.3%, *T. coccinea* and *T. tagusensis* respectively) in hexane:DCM fractions. The substance palmityl oleate (C_{34}) was found only in hexane fraction (24% *T. coccinea* and 19.2% *T. tagusensis*). On the other hand, the DCM fractions

showed a diverse array of sterols (C_{26} – C_{29}) and cholest-5-en-3β-ol (cholesterol) had the highest values observed in *T. coccinea* (42.3%) and *T. tagusensis* (55.3%). The dominant compound cholesterol was also observed in *T. coccinea* (100%) and *T. tagusensis* (68.8%) in the DCM:MeOH fractions (Table 1). The hydrocarbon 1-hexadecene was observed in the hexane:DCM (15.5%) and DCM:MeOH (31.2%) fractions from *T. tagusensis* (Table 1).

One of the replicate treatments used in the field experiment was lost. Five compounds, in each treatment, contained straight chain hydrocarbons (C_{16} to C_{22}) and were tentatively (>90% probability) identified by GC/MS, as 1-hexadecene, *n*-hexadecane, *n*-octadecane, *n*-eicosane and *n*-docosane (Table 2). Despite the fact that 1-hexadecene was found higher concentration in different fractions of the MeOH extract of *Tubastraea tagusensis* (Table 1), it represented 7.2% and 5.2% of the total composition of substances detected in control and treatment in field experiment, respectively; *n*-octadecane appeared in higher concentrations (37.9% and 39.7%, respectively) (Table 2). The contents of 1-hexadecene, *n*-hexadecane and *n*-eicosane showed significant differences between control and treatment (see Table 2). *n*-docosane tended to be more abundant in treated than control extracts but analysis did not detect significant differences between control and treatment ($p = 0.076$; Mann-Whitney U test).

Table 1. GC-MS analysis of % area of *n*-hexane, *n*-hexane:DCM, DCM and DCM:MeOH fractions from MeOH extracts for *T. coccinea* (T.C.) and *T. tagusensis* (T.T.).

M[1]	Molecular formula	Chemical substances[2]	*n*-hexane fraction (Area %)		*n*-hexane:DCM fraction Area (%)		DCM fraction Area (%)		DCM:MeOH fraction Area (%)	
			T.C.	T.T.	T.C.	T.T.	T.C.	T.T.	T.C.	T.T.
224	$C_{16}H_{32}$	1-hexadecene	-	-	0.4	15.5	2.2	-	-	31.2
240	$C_{16}H_{32}O$	hexadecanal	-	-	2.1	-	-	-	-	-
242	$C_{15}H_{30}O_2$	methyl myristate	-	1.0	-	-	-	-	-	-
252	$C_{18}H_{36}$	1-octadecene	-	-	1.5	9.4	2.1	-	-	-
268	$C_{17}H_{32}O_2$	methyl palmitoleate	-	2.1	3.1	-	-	-	-	-
270	$C_{17}H_{34}O_2$	methyl palmitate	17.1	19.6	18.0	21.3	-	-	-	-
282	$C_{18}H_{34}O_2$	oleic acid	1.5	1.9	1.4	-	-	-	-	-
284	$C_{18}H_{36}O_2$	methyl margarate	1.6	2.0	1.6	-	-	-	-	-
296	$C_{19}H_{36}O_2$	methyl oleate	13.1	14.8	17.2	17.0	-	-	-	-
298	$C_{19}H_{38}O_2$	methyl stearate	20.3	28.1	23.6	29.2	-	-	-	-
312	$C_{20}H_{40}O_2$	methyl n-nonadecanoate	1.4	2.0	1.5	-	-	-	-	-
324	$C_{21}H_{40}O_2$	methyl 11-eicosenoate	2.5	2.5	4.0	-	-	-	-	-
326	$C_{21}H_{42}O_2$	methyl arachate	2.6	2.7	3.3	-	-	-	-	-
352	$C_{23}H_{44}O_2$	methyl erucate	-	1.4	1.7	-	-	-	-	-
370	$C_{26}H_{42}O$	26,27-dinorergosta-5,22E-dien-3 β-ol	-	-	-	-	2.2	1.7	-	-
384	$C_{27}H_{44}O$	27-norergosta-5,22-dien-3β-ol	-	-	-	-	15.2	17.3	-	-
384	$C_{27}H_{44}O$	cholest-5-en-3β-one	-	-	-	-	0.4	-	-	-
386	$C_{27}H_{46}O$	cholest-5-en-3β-ol	-	-	-	-	42.3	55.3	100.0	68.8
398	$C_{28}H_{46}O$	(22E,24S)-crinosterol	-	-	-	-	4.6	-	-	-
398	$C_{28}H_{46}O$	22,23-ciclopropane-cholesterol	-	-	-	-	19.5	-	-	-
398	$C_{28}H_{46}O$	ergosta-5,22-dien-3β-ol	-	-	-	-	-	5.4	-	-
398	$C_{28}H_{46}O$	22,23-metilene-cholesterol	-	-	-	-	-	11.1	-	-
400	$C_{28}H_{48}O$	campesterol	-	-	-	-	-	5.5	-	-
400	$C_{28}H_{48}O$	23-R-methylcholesterol	-	-	-	-	3.7	-	-	-
412	$C_{29}H_{48}O$	stigmasta-5,22E-dien-3β-ol	-	-	-	-	1.4	-	-	-
412	$C_{29}H_{48}O$	stigmasta-5,24(28)-dien-3β-ol	-	-	-	-	1.6	3.8	-	-
414	$C_{29}H_{50}O$	stigmast-5-en-3β-ol	-	-	-	-	5.1	-	-	-
478	$C_{32}H_{62}O_2$	myristyl oleate	-	-	-	0.48-	-	-	-	-
506	$C_{34}H_{66}O_2$	cetyl oleate	-	-	5.9	-	-	-	-	-
506	$C_{34}H_{66}O_2$	palmityl oleate	24.05	19.17	-	-	-	-	-	-
508	$C_{34}H_{68}O_2$	stearyl palmitate	3.30	2.78	-	-	-	-	-	-
534	$C_{36}H_{70}O_2$	stearyl oleate	12.7	-	15.5	7.1	-	-	-	-

[1]Molecular weight and [2]Tentatively identified (> 90% probability) by co-injections with authentic samples and Wiley 275 mass spectra library.

Table 2. Mean proportion of substances detected by GC-MS analysis of DCM extracts from seawater near injured and control colonies of *T. tagusenisis* captured in Sep-Paks® cartridges in an *in situ* experiment. Comparison between control and treatment concentration was by nonparametric Mann-Whitney U test (Z).

M[1]	Molecular formula	Chemical substances[2]	Control mean area (%)	Treatment mean area (%)	Z	Significance (*p*)
224	$C_{16}H_{34}$	1-hexadecene	7.2 (± 0.41 SE)	5.2 (± 0.48 SE)	-2.611	0.009*
226	$C_{16}H_{32}$	*n*-hexadecane	25.1 (± 6.28 SE)	7.9 (± 2.57 SE)	-2.402	0.016*
254	$C_{18}H_{38}$	*n*-octadecane	37.9 (±0.59 SE)	39.7 (± 1.64 SE)	-0.940	0.347
282	$C_{20}H_{42}$	*n*-eicosane	19.6 (± 4.27 SE)	32.6 (± 2.59 SE)	-2.611	0.009*
310	$C_{22}H_{46}$	*n*-docosane	10.2 (± 1.43SE)	14.6 (± 1.79 SE)	-1.776	0.076

[1]Molecular weight; [2]Tentatively identified (> 90% probability) by co-injections with authentic samples and Wiley 275 mass spectra library; SE = standard error; *significant difference.

DISCUSSION

Many metabolites produced by cnidarians are assumed to act against predators and competitors (COLL, 1992; PAWLIK, 1993; HARPER et al., 2001; LAGES et al., 2006; PAUL et al., 2006; FLEURY et al., 2008). The phylum Cnidaria possesses an array of secondary metabolites, mainly terpenes, and the soft coral group possesses more than 80 percent of all cnidarian compounds (HARPER et al., 2001; BLUNT et al., 2007, 2008). Despite the absence of accessible fleshy and soft tissues, scleractinian corals also produce secondary metabolites for defense and many of them are toxic to cells (FUSETANI et al., 1986; GUELLA et al., 1988; FUNG et al., 1997, KONTIZA et al., 2006); predators (BAIRD et al., 2001) and competitors (DE RUYTER VAN STEVENICK et al. 1988; KOH; SWEATMAN, 2000).

The detection of fatty acids and sterols by GC-MS showed that *Tubastraea* spp produced several secondary metabolites that may act antagonistically to other organisms and thus be responsible, in part, for success in invasion on rocky shores at Ilha Grande Bay (LAGES et al., 2010). Sterol composition, mainly cholesterol, of a variety of marine invertebrates has been investigated in detail (KANAZAWA, 2001; BLUNT et al., 2009). Yamashiro et al. (1999) identified 11 major sterols common to 15 species of cnidarians including scleractinian corals. According to these authors, cholesterol was the predominant substance for *Tubastraea* sp. The major substance found in our DCM fraction for *T. tagusensis* was also cholesterol, where it represented more than 50% of the substances present. A range of sterols isolated from soft corals and gorgonians have exhibited biological and ecological activities (TOMONO et al., 1999; EPIFANIO et al., 2007; CHANGYUN et al., 2008). Slattery et al. (1997) studied metabolites in the soft corals *Alcyonium paessleri* and *Gersemia antarctica*, which were mainly sterols identified as cholesterol, 22-dehydro-cholesterol, 24-methylene-cholesterol, 22-dehydro-7β-hydroxy-cholesterol released into the surrounding seawater and demonstrated that these compounds played predator deterrence and antibacterial roles, respectively.

All substances from different extracts, mainly sterols, fatty acids and hydrocarbons detected by GC-MS here have been registered in corals in other studies (YAMASHIRO et al., 1999; HARPER et al., 2001; CHANGYUN et al., 2008; PAUL; RITSON-WILLIAMS, 2008; BLUNT et al., 2007, 2008, 2009). Despite the hydrocarbons detected here in field experiment, it seems that the other classes of substances are not appreciably water soluble as their absence in Sep-Paks® was confirmed by GC-MS. Furthermore, scleractinian corals possess low fresh tissue/skeleton ratio compared with soft corals which lowers the volumetric concentration of substances inside the coral and may reduce sterols and fatty acids in seawater to undetectable levels.

It is known that allelochemical compounds produced by some soft corals can help them to expand or avoid competitors such as scleractinian corals and other soft corals (COLL et al., 1982; SAMMARCO et al., 1985; LA BARRE et al., 1986; COLL, 1992; MAIDA et al., 1995; SLATTERY et al. 1997; KOH; SWEATMAN, 2000; MAIDA et al., 2001; FLEURY et al., 2004; 2006). The exotic coral *Chromonephthea braziliensis* (Alcyonacea) has established in Brazil and also produces a range of secondary metabolites (hemiketal steroid, 23-keto-cladiellin-A) which help protect this soft coral from predators (FLEURY et al., 2008) and in its competition for space (FERREIRA, 2003; LAGES et al., 2006). Giner and cols. (2008) found polyunsaturated fatty acids in an alga, previously classified in the genus *Chattonella* as

responsible for ictyotoxic effects around the world. Jüttner (2001) showed that diatom biofilms produced polyunsaturated fatty acids which can also act as defense substances against grazers. In fact, a range of fatty acids produced by algae exert negative effects on a variety of aquatic organisms (SPRUELL, 1984; IKAWA et al., 1996; KAMAYA et al., 2003; MUNDT et al., 2003). Therefore, numerous researchers have suggested that fatty acids might also serve as allelochemicals in aquatic organisms (KAKISAWA et al., 1988; SUZUKI et al., 1996; CHIANG et al., 2004). Hydrocarbons, fatty acids and esters are considered as allelopathic agent in plants (GEORGIEVA et al. 2005). Therefore, compounds produced by *Tubastraea*, may be aiding this exotic species to expand in the new area, even though there are no known specialist predators in this region.

Lages and cols. (2010) demonstrated experimentally that *Tubastraea coccinea* and *T. tagusensis* produce chemical substances which can affect negatively interactions with potential predators and foulers. Creed (2006) showed also that when in close proximity *Tubastraea* caused necrosis in the endemic scleractinian coral *Mussismilia hispida*. This evidence so far suggests that these species produce active compounds used against competitors. The harmful effects produced by chemical compounds of *Tubastraea* on other organisms together with its reproductive strategy brings advantages to the corals (GLYNN et al., 2007) and could explain their success in invading new regions to the detriment of local fauna and flora.

It is known that the mucus produced by corals possesses an array of esters, sterols and other lipids, including hydrocarbons, some of which are soluble in seawater at low concentrations (BENSON; MUSCATINE, 1974; NAKAJIMA et al., 2009). Considerable levels of straight-chain hydrocarbons produced by corals and other marine organisms are products of coral lipogenesis, rather than derived from dietary sources (CLARK; BLUMER, 1967; MEYERS, 1977). Contrary to unsaturated or oxygenated compounds such as sterols and esters that can be easily oxidized by hypochlorite (bleach), saturated hydrocarbons do not react, and thus are not solubilized. Therefore, hydrocarbons were found in the control (skeleton) through GC/MS. On the other hand, the high concentration of 1-hexadecene and n-hexadecane on Sep-Paks® control compared with treatments, detected by GC-MS, was due to their great affinity for seawater. Other exuded substances such as *n*-eicosane may be bound on surface tissues or in mucus and thus would show low concentrations in waterborne samples of the control.

Coll et al. (1982) demonstrated that the concentration of cembranolides terpenes (allelopathic compounds) in the sea water around the soft corals *Sinularia flexibilis* and *Sarcophyton crassocaule* was quite low (1 to 5 ppm). We, therefore, suggest that the concentration of substances around *T. tagusensis* trapped by Sep-Paks® cartridges was probably also very low. These compounds were thus detected and confirmed by GC-MS, but not detected by TLC.

This experiment created an artificial injury where hydrocarbons were released and this leads to the hypothesis that interference competition (JACKSON; BUSS, 1975) and mechanisms of interspecific aggression such as predation between species of marine organisms may be linked to the excretion of toxic substances (COLL et al., 1982). Although not conclusive, the presence of the substances in Sep-Paks® columns coupled with the probable toxicity (allelopathic action) of hydrocarbons (according to the study cited above) suggests a negative interactive mechanism. The next step would be to detect variability in chemical compounds of *Tubastraea* spp due to the proximity of potential competitors in the field to confirm this hypothesis.

The exotic corals *T. coccinea* and *T. tagusensis* have established and invaded rocky reefs at Ilha Grande Bay (PAULA; CREED, 2004), being most abundant in the shallow subtidal depths where they reach very high densities compared to native species (PAULA; CREED, 2005). The present study has described the chemical compounds produced by these exotic corals and the application of a novel method which detected chemical compounds after injury to the corals *in situ*. The mediation of negative interactions through chemical compounds and the high abundance of the exotic invasive corals on rocky shores of Ilha Grande Bay is therefore expected to increase the frequency of these negative chemical interactions with the native benthic communities over time and will lead to further change in community structure as well as facilitate further range expansion of the exotic corals.

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