Spatio-temporal variability of secondary metabolites in the invasive coral *Tubastrea coccinea*

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**Abstract**

*Tubastrea coccinea* is a coral species originally described for the Pacific Ocean. It is widely distributed throughout the Brazilian coast and in several other Atlantic areas. Its widespread occurrence is presumably facilitated by its production of secondary metabolites with defensive action against predators and competitors. This study evaluated the spatial and temporal variability in the chemical profile of *T. coccinea* in Arraial do Cabo Bay, Southeastern Brazil, using GC/MS analysis. We also compared the profiles between sites/seasons with non-Metric Multidimensional Scaling (n-MDS) and Principal Component Analysis (PCA). Our results showed that the total metabolite (extract yield) decreased in winter and increased significantly in spring. Sterols and fatty acid esters were the main compounds identified in the four *T. coccinea* populations. The extracts differed qualitatively and quantitatively between the four *T. coccinea* populations. Winter samples had the highest lipid contents, although they showed the lowest contents of total metabolites. The highest values of extract yields were obtained for the spring samples, while in fall it did not show significant differences. The n-MDS and PCA also revealed differences in the chemical profiles between fall, winter, and spring samples. However, the observed chemical variability did not allow a clear distinction between the *T. coccinea* populations. It did reflect similar environmental conditions at sites close to the ocean and sheltered areas. This invasive coral has already adapted itself to the presumed dynamics of interactions with competitors and consumers.

**Key words:** sun coral, non-indigenous species, chemical profile, marine natural products, metabolomic, multivariate analyses, chemometric tool

**Introduction**

The introduction of non-indigenous species (NIS), which can eventually become invasive, is recognized as one of the main threats that can promote significant changes in the structure and dynamic of marine ecosystems (Costello et al. 2010; Simberloff et al. 2013). Various marine biomes, such as coral reefs, algal meadows, mangroves and seagrass beds (Salimi et al. 2011).
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2021) are threatened by the introduction of non-indigenous species, contributing to the declines of native species (Gurevitch and Padilla 2004) and expressive cascade effects on biodiversity, ecosystem functions, and trophic levels (Anton et al. 2019). Their impacts can vary at small (Ceccherelli and Campo 2002) and regional spatial scales (Bulleri et al. 2010) or have broader effects, such as between seasons (Scheibling and Gagnon 2006) and across years (Staehr et al. 2000).

One of the greatest challenges of biological invasions is knowing what makes certain species invasive in a new habitat and how bioinvasion processes occur (Geburzi and McCarthy 2018). However, some general aspects are recurrent, such as common invasion pathways and vectors (Katsanevakis et al. 2012), anthropogenic disturbance of the invaded habitats (Bax et al. 2000; Briggs 2012; Mineur et al. 2012) as well as the ecology, physiology, evolution, and genetics of the invader organisms (Geburzi and McCarthy 2018). Furthermore, secondary metabolites production by NIS is hypothesized as a strategy to colonize, establish, and spread to new areas (Pereira 2004). Some studies have corroborated this hypothesis for coral species (e.g. Lages et al. 2006) and, since corals produce a great richness and diversity of these chemicals, NIS corals are potentially invasive species.

For example, in the soft coral Parerythropodium fulvum fulvum, the concentrations of fulfulvene, the major metabolite in yellow morph, and of calamine in their grey morph were significantly different between shallow and deep colonies (Kelman et al. 2000). In Sinularia flexibilis colonies, the concentrations of the two terpenoids flexibilide and sinulariolide were significantly different between the three sites where the coral was found (Maida et al. 1993). During a two-year monitoring, Slattery et al. (2001) observed that the concentrations of two major pukalide and 11β-acetoxypukalide, the two primary defensive metabolites in the soft corals Sinularia maxima and S. polydactyla, showed a significant temporal variation, but without a seasonal pattern. In addition, the temperature was the main factor that influenced the fatty acid profiles in studies carried out with the scleractinean corals Acropora millepora and Goniastrea aspera for one year (Conlan et al. 2020; Oku et al. 2003).

Species of Tubastrea, popularly known as “sun corals”, produce a wide variety of secondary metabolites, including alkaloids (Fusetani et al. 1986; Iwagawa et al. 2003, 2008), macrolides (Rashid et al. 1995), and sesquiterpenes (Alam et al. 1988). Steroids, fatty acids, and fatty acid derivatives (Carpes et al. 2020; Lages et al. 2010b), carotenoids (Maia et al. 2014), and alkaloids (Okuda et al. 1982) have also been found in the metabolite profile of T. coccinea. Some of these compounds were demonstrated as a defense mechanism against competitors (Koh and Sweatman 2000; Lages et al. 2010a, 2012), which has helped maintain its great abundance, causing disturbances in native benthic communities (Lages et al. 2011). Tubastrea
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*Tubastrea coccinea* is probably the most invasive alien coral reported to date, capable of settling on various substrate types (Fenner 2001; Fenner and Banks 2004; De Paula and Creed 2004; Sammarco et al. 2004; López et al. 2019). Its success is presumably due to its powerful defensive chemical arsenal (Lages et al. 2015).

*Tubastrea coccinea* is native to shallow waters of the Indo, Western and Central Pacific, with a cryptogenic origin in the Eastern Atlantic, also introduced in the Atlantic Ocean. This species is considered cosmopolitan since it has spread worldwide, from tropical (Fenner 2001; Fenner and Banks 2004) to temperate zones (Cairns and Zibrowius 1997; Paz-García et al. 2007). It has been described as an invasive species in some places (Brito et al. 2017), which includes the Western Atlantic areas (Creed et al. 2017). *Tubastrea coccinea* is widespread in the Brazilian coast (De Paula and Creed 2004), with several disjunct populations distributed along the 3,800 km of coastline, from the Northeast (Ceará state, Soares et al. 2016) to the South (Santa Catarina state, Capel 2012). In the Southeastern Brazil, an upwelling occurs with high frequency and intensity between spring and summer months, specifically in the city of Arraial do Cabo (Calado et al. 2008; Castelao and Barth 2006; Santos et al. 2019). Studies with *T. coccinea* carried out in Arraial do Cabo Bay showed that this phenomenon exerts a control interaction in the expansion and survival of its colonies and recruits. Higher densities have been observed in areas with high water temperatures (> 20 °C), being absent in areas strongly influenced by cold upwelling waters (Batista et al. 2017).

In this study, we aimed to assess the chemical profile of four populations of *T. coccinea* in the fall, winter, and spring seasons. We hypothesized that the chemical profiles of *T. coccinea* would vary on spatial and temporal scales in the Arraial do Cabo Bay region, where this species was introduced and established.

**Materials and methods**

**Study area and collections of T. coccinea**

Arraial do Cabo is a coastal tropical/subtropical area with rich and biodiverse reefs (e.g. De Paula et al. 2020), composed of different components, such as a diverse epilithic algal community (Guimaraens and Coutinho 1996), *Palythoa caribaeorum* patches, *Millepora alcicornis* colonies, and four species of hermatypic corals (Castro et al. 1995), as well as sponges, ascidians, bryozoans, and hydrozoans.

In the studied area, *T. coccinea* colonies are commonly distributed in patches on rocky shores. They exhibit the highest density in Ilha dos Porcos and similar and intermediate densities in Enseada do Anequim and Pedra Vermelha. The lowest density is found in Saco dos Cardeiros, within Arraial do Cabo Bay (Batista et al. 2017). This coral does not occur in regions...
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**Figure 1.** Map of Arraial do Cabo Bay (Rio de Janeiro, Brazil) indicating the sampling sites. Area 1 (blue – sheltered portion of the Bay): SC = Saco dos Cardeiros; IP = Ilha dos Porcos. Area 2 (red – exposed area): EA = Enseada do Anequim; PV = Pedra Vermelha. The arrow indicates the incoming water flow from the Atlantic Ocean into the Bay.

Coral specimens were sampled from shallow waters at four different sites in the Marine Extractive Reserve located within Arraial do Cabo Bay (Enseada do Anequim, 22°58′51″S; 41°59′00″W, Saco dos Cardeiros, 22°57′56″S; 42°00′08″W, Ilha dos Porcos, 22°58′01″S; 41°59′33″W, and Pedra Vermelha, 22°59′16″S; 41°59′34″W), Rio de Janeiro state, southeastern Brazil (Figure 1). These locations were distinguished as closer to the inner and outside portions of the Bay (Area 1 and 2, respectively) according to Batista et al. 2017. Therefore, Saco dos Cardeiros and Ilha dos Porcos belong to Area 1 (at the mainland and Porcos Island), and Pedra Vermelha and Enseada Anequim belong to Area 2 (at the Cabo Frio Island). In total, twelve samples were collected in 2018 by SCUBA diving: one for each sampling site in fall (April), winter (August), and spring (beginning of December) at a depth of 5.0–6.0 m.

**Extraction procedures**

Colonies of *T. coccinea* were extracted according to common methods previously described (Lages et al. 2010b), with few modifications. Samples were first lyophilized and then extracted with a mixture of ethyl acetate and methanol (EtOAc: MeOH 1:1 v/v), at the proportion of 4.14 mL of solution per 1g of dry weight (dw) sample, and sonicated for 20 minutes.
The extraction was carried out with a standing time of 2 h period at room temperature, posteriorly filtered, and concentrated under reduced pressure. This procedure was repeated three times for each sample to obtain the final extract yield (extract weight/dw). Twelve crude extracts were obtained (one per each sampling site/season) which were subsequently analyzed by Gas Chromatography coupled to Mass Spectroscopy (GC/MS) to evaluate their chemical profiles.

**GC/MS conditions**

The extracts obtained were analyzed by gas chromatography-mass spectroscopy (GC/MS) to evaluate the chemical profile variability of *T. coccinea*. Prior to GC/MS injection, each extract was diluted in dichloromethane (HPLC, Tedia), and filtered using a 0.45-μm PTFE syringe filter (Millipore, USA) to remove any insoluble constituent. The solvent was evaporated, and the samples were subsequently lyophilized overnight to eliminate water (humidity). The remaining material was resuspended in ethyl acetate (HPLC grade, Tedia) to a final concentration of 1 mg/mL.

Analysis by GC/MS was carried out in a GC-2010 plus SHIMADZU coupled to a mass spectrometer QP-2010 ultra, composed by an autoinjector AOC-20i, with electron impact ionization at 70 eV and column Rtx-1MS 30 m × Øint 0.25 mm. The column flow rate was 1.20 ml/min (AcOEt solution), injected in a split mode ratio (1/5), and helium as carrier gas. The oven temperature was programmed from 160 °C for 3 minutes, followed by three temperature ramps, being the first one up to 260 °C, the second one from 260 °C to 300 °C, and the last one from 300 °C to 310 °C, totaling 31 minutes. Injector and ions source temperature was kept constant at 280 °C and 200 °C, respectively. Samples were injected and analyzed in duplicate.

The chemical profiles were compared based on mass spectra data and retention time. The compounds were identified by comparing the mass spectra of each substance with those available in the NIST05 library when they exhibited a similarity index higher than 90%, followed by values of percentage of the relative area (Supplementary material Table S1). Substances with abundance greater than 500000 uV were considered major compounds (Figures S1, S2 and S3). The same numbers and letters represent the same substance in the samples. Numbers represent the identified substances in the Table S1 (SI > 90%) and letters the non-identified substances (SI < 90%).

**Data analysis**

A two-way analysis of variance (ANOVA) with random effect was used to compare the percentage of extraction yields between samples. A *post-hoc* Tukey’s test was performed for paired comparisons, and the results were considered significant when *p* ≤ 0.05.
Multivariate analyses were performed to investigate the possible chemical profile variability in samples of *T. coccinea* samples. The COWtool software (http://www2.biocentrum.dtu.dk/mycology/analysis/cow/) was used for base alignment of each chromatogram and to correct the retention time of the peak shifts through the correlation-warping (COW) algorithm (see Nielsen et al. 1998). A matrix with all aligned chromatograms was constructed and used for the non-metric multidimensional scaling (n-MDS) and Principal Component Analysis (PCA). Initially, to elucidate the chemical profile similarities and differences in the low-dimensional space, the data were subjected to n-MDS using PAST software. The data matrix with square root transformation was used to create the triangular similarity matrices based on the Bray-Curtis similarity coefficient, followed by n-MDS. In this sense, the robustness of n-MDS grouping patterns in differentiating the chemical profile was assessed by two-way analysis of similarity (ANOSIM) with 9999 permutations. For all tests, the results were considered significant when \( p \leq 0.05 \). Posteriorly, a PCA was applied as an exploratory chemometric tool to visualize the principal compounds responsible to discriminate the observed groups. The matrix with the untransformed data was centralized, and the PCA was run using the “ChemometricsWithR” package (Wehrens 2011) in R (http://www.R-project.org). Redundant variables did not assist in understanding the total variance. Thus, for better visualization of results, we removed the four major compounds found in all samples from the dataset: oleic acid hexadecyl ester, oleic acid eicosyl ester, (3β)-cholesta-5,22-dien-3-ol and (3β)-cholest-5-en-3-ol (see peaks 30–33, Table S1). The PCA results were represented by scatter plots of the scores of the Principal Components for visualization of the discriminant pattern of the samples.

**Results**

*Extracts yields*

Whether analyzing the variability in the extraction yields, any concerted interaction effect was found in the metabolic production among seasons and collection sites (Table 1; Two-way ANOVA, \( p > 0.02 \)). However, a significant differentiation in the extract yield between samples from different seasons (Figure 2a, Table 1, Two-way ANOVA, \( p = 0.02 \)). The highest mean extract yield was found in spring samples (4.64 % ± 0.96), followed by fall (4.06 % ± 0.79) and winter (2.95 ± 0.61). In contrast, the collection sites were not determinant for a variability in the amount of extracted metabolites content (Figure 2b, Table 1, Two-way ANOVA, \( p = 0.40 \)).

*Chemical profile characterization of T. coccinea*

In total 46 compounds were found in *T. coccinea* extracts from Enseada do Anequim (EA), Saco do Cardeiros (SC), Ilha dos Porcos (IP) and Pedra
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**Table 1.** Statistical summary of the results from two-way ANOVA with random effect. Values in bold indicate statistically significant results.

<table>
<thead>
<tr>
<th></th>
<th>Degrees of Freedom (DF)</th>
<th>Sum of Squares (SS)</th>
<th>Mean Square (MS)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
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<td>5.84</td>
<td>2.92</td>
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</tr>
<tr>
<td>Collection areas</td>
<td>1</td>
<td>0.32</td>
<td>0.32</td>
<td>0.82</td>
<td>0.40</td>
</tr>
<tr>
<td>Season * Collection areas</td>
<td>2</td>
<td>3.10</td>
<td>1.54</td>
<td>3.94</td>
<td>0.08</td>
</tr>
<tr>
<td>Residual</td>
<td>6</td>
<td>2.35</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>11.61</td>
<td>1.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2.** Mean and standard deviation of the percentage of the extraction yields obtained from colonies of *T. coccinea* by seasons (a). Mean and standard deviation of the percentage of the extraction yields of colonies of *T. coccinea* analyzed by region (Area 1 – sheltered portion of the Bay: SC and IP, Figure 1; Area 2 – exposed area, EA and PV, Figure 1 (b).

Vermelha (PV) in fall, winter, and spring samples (Table S1). In general, the chemical profiles of all extracts evidenced compounds with long carbon chains (C₄-C₉), which comprised three hydrocarbons, eight alcohols, eleven aldehydes, three fatty acids, two sterols, ten nitrogen compounds, and three other unclassified chemical types, based on identification by mass spectra data. As shown in Table S1, the most abundant peaks were annotated as (3β)-cholesta-5,22-dien-3-ol (Peak 32), (3β)-cholest-5-en-3-ol (Peak 33), oleic acid hexadecyl ester (Peak 30) and oleic acid eicosyl ester (Peak 31). These compounds were observed in the colonies from all collection sites (EA, SC, IP and PV) and seasons. Six other unidentified substances (SI < 90%) were also classified as major compounds (Figures S1, S2 and S3). The minor compounds had a very irregular seasonal and site occurrence since they were only present in a few *T. coccinea* extracts. However, although the winter colonies exhibited the lowest metabolic production (Figure 2a), they showed the greatest diversity of metabolite classes, with 70% nitrogen compounds, 87.5% alcohols, and the highest concentrations of major fatty acid esters (Peaks 30–31, Table S1).

**Multivariate analysis of the chemical profile variability**

The n-MDS ordination analysis showed chemical variability between *T. coccinea* samples (Figure 3), corroborating the results of the extraction yield.
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Figure 3. Non-metric multidimensional scaling plot of the metabolic content of Tubastrea coccinea samples grouped by seasons: spring in pink, fall in brown and winter in blue (a) and sampling area: Area 1 in gray – inner portion of the Bay, SC and IP; Area 2 in black– portions with higher exposure to the open ocean, EA and PV (b). Analysis based on Bray-Curtis dissimilarities through a square-root transformation of the abundance values of the crude extracts metabolic composition (stress value = 0.18). EAF = Enseada do Anequim Fall, EAW = Enseada do Anequim Winter, EAS = Enseada do Anequim Spring, IPF = Ilha dos Porcos Fall, IPW = Ilha dos Porcos Winter, IPS = Ilha dos Porcos Spring, PVF = Pedra Vermelha Fall, PVW = Pedra Vermelha Winter, PVS = Pedra Vermelha Spring, SCA = Sacos dos Cardeiros Autumn, SCW = Saco dos Cardeiros Winter, SCS = Saco dos Cardeiros Spring.

Table 2. Statistical summary of the results from Two-way ANOSIM test of T. coccinea chemical profiles through the factors season and collection areas comparisons. Values in bold indicate statistically significant results.

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>p</th>
<th>Number of permutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>0.39</td>
<td>0.02</td>
<td>9999</td>
</tr>
<tr>
<td>Population</td>
<td>0.33</td>
<td>0.22</td>
<td>9999</td>
</tr>
</tbody>
</table>

Figure 3a revealed three distinct groups corresponding to the chemical profile of fall, winter, and spring extracts. The stress value obtained (Figure 3, stress value = 0.18), provides a good representation/ordination of the data in reduced dimensions (Dexter et al. 2018). Two-way ANOSIM test confirmed the existence of a significant difference between samples compared seasonally (Table 2, R = 0.39; p = 0.02). In contrast, in the analysis by collection sites (Figure 3b), despite a good separation between the two sampling sites, collections close the open ocean (PV and EA, Figure 1) and and within more sheltered area (IP and SC, Figure 1), it did not show significant differences in relation to production of metabolites (Table 2, Two-way ANOSIM, R = 0.33; p = 0.22).

Correspondingly, the PCA (Figure 4) showed a chemical similarity between samples according to the collection sites (PC1) and seasonality (PC3). The first three principal components explained 58.2% of the variability. PC1 explained 42.0% of the chemical variability, contributing most to understanding the metabolite variations. In agreement with the previous analyses, two groups were discriminated. The negative PC1 grouped the Area 1 samples (PV and EA, Figure 1) collected close to the open ocean. In contrast, the positive PC1 grouped the extracts from the northern sheltered Area 2 samples (IP and SC, Figure 1), except for one sample.
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**Figure 4.** Ordination graph of the first and third PCA scores (PC1 and PC3) obtained from the analysis of the chemical variability in colonies of *T. coccinea*. EAF = Enseada do Anequim Fall, EAW = Enseada do Anequim Winter, EAS = Enseada do Anequim Spring, IPF = Ilha dos Porcos Fall, IPW = Ilha dos Porcos Winter, IPS = Ilha dos Porcos Spring, PVF = Pedra Vermelha Fall, PVW = Pedra Vermelha Winter, PVS = Pedra Vermelha Spring, SCF = Saco dos Cardeiros Fall, SCW = Saco dos Cardeiros Winter, SCS = Saco dos Cardeiros Spring.

Three trends were observed considering the third component. Spring extracts were found mainly on the negative PC3 axis, while most of the winter samples were grouped on the positive side of the same axis, except for EAW. The fall samples were also closely distributed, despite exhibiting greater dispersion in the ordination plot, supporting previous results. Tributyl acetylcitrate (Peak 28, Table S1) was the main compound that contributed positively to PC1. In contrast, an unidentified sterol and four unidentified fatty acids were the primarily responsible for the negative contribution to PC1. Unlike PC1, tributyl acetylcitrate contributed negatively to PC3, and octadecanal (Peak 21, Table S1) had the greatest contribution on the positive PC3 axis.

GC/MS analysis showed that fall and spring colonies produced tributyl acetylcitrate (Peak 28, Table S1). This compound has already been identified in extracts of fungi isolated from soil (Neveen et al. 2014) and marine sponges (Binh et al. 2018). The compounds 1-nonadecene (Peak 3, Table S1) and hexanedioic acid bis(2-ethylhexyl) ester (Peak 29, Table S1), showed the same trend, being absent in all *T. coccinea* colonies from winter.
samples. However, 1-nonadecene (Peak 3, Table S1) was identified in six fall extracts (Enseada do Anequim and Ilha dos Porcos) and all spring samples (EA, SC, IP and PV). The hexanedioic acid bis(2-ethylhexyl) ester (Peak 29, Table S1) was found in two fall samples (SC and IP) and all spring samples (EA, SC, IP and PV). Both compounds (Peaks 3 and 29, Table S1) did not contribute to the PCA results.

Discussion

This study found qualitative and quantitative temporal and/or seasonal variability in the chemical profiles of secondary metabolites produced by colonies of the invasive coral *T. coccinea* in Arraial do Cabo Bay, southeastern Brazil. Furthermore, although without significant relevance, the populations from areas more exposed to the open ocean (Pedra Vermelha and Enseada do Anequim) differed metabolically from those found in more sheltered areas (Ilha dos Porcos and Saco dos Cardeiros). A larger number of samples from each collection site would perhaps reveal significant differences between these populations.

Colonies of *T. coccinea* from the four collection sites had the highest mean amounts of extract in spring, while winter extracts showed the lowest total secondary metabolites. However, the highest metabolite production at each sampling site was achieved in a given season. For instance, Enseada do Anequim, and Pedra Vermelha colonies attained the highest percentage of extract content in fall. In contrast, Ilha dos Porcos de Saco dos Cardeiros had the highest values in winter, and Pedra Vermelha in spring. The lack of statistical significance in metabolite production differentiation between collection sites may have been limited by the experimental design employed in this study. Perhaps through a better experimental design including a greater number of replicates, and/or more populations (repetition) it would be possible to better validate the results obtained (see Vaux et al. 2012; Blainey et al. 2014).

The mean amount of *T. coccinea* extract at each site and season could express this species’ presumably dynamic or variable relationship with environmental conditions and/or consumers and competitors, on which the evolution of the chemical interactions occur (Hay 1996). For example, areas with higher field temperatures showed higher *T. coccinea* density and recruitment. Lower temperatures negatively affected the survival of this coral in laboratory tests (Batista et al. 2017), and presumably its defensive chemistry against consumers. In marine organisms, extract levels have been used in comparative geographic approaches of allelopathic efficiency since higher levels would have a more effective defensive performance (e.g. Bolser and Hay 1996; Becerro et al. 2003). Because extracts of *T. coccinea* exhibit defensive properties (Koh and Sweatman 2000; Lages et al. 2010a, 2012), we could assume that this coral would be susceptible differently to natural enemies (e.g. competitors and predators) throughout the year and
Secondary metabolites variability in *Tubastrea coccinea* particularly to environmental pressures within the region studied (Coelho-Souza et al. 2012). These assumptions corroborate with the extract content variation observed in the present study and the groupings that exhibit variability in the chemical profile formed by the multivariate analyses.

On the Brazilian coast, the native sponge *Desmapsamma anchorata* has been described as a potential competitor of *Tubastraea* species, overgrowing and killing *T. coccinea* and *T. tagusensis* populations in Ilha Grande Bay (Meurer et al. 2010) and could serve for biological control against the invasive corals (Silva et al. 2022). Despite being commonly found in the states of Bahia and Rio de Janeiro, *D. anchorata* has not yet been reported in the study area (Soares et al. 2016). On the other hand, the highly toxic and native zoanthid *Palythoa caribaeorum* is also a competitor of *Tubastraea* species and has suffered chemical and physical injuries by *T. coccinea* and *T. tagusensis* (Luz and Kitahara 2017; Almeida Saá et al. 2020). Unlike *D. anchorata*, *P. caribaeorum* is widely distributed within the Arraial do Cabo Bay (Freret-Meurer and Oliveira 2012; Rogers et al. 2014), supporting the assumption that the chemical profile variation in *T. coccinea* colonies studied could be related to competition interactions. In the context of predators, methanol extract of *T. coccinea* did not inhibit fish communities in the natural environment, but *T. tagusensis* extract acted as a chemical defense (Lages et al. 2010a). However, this possibility of defensive action produced by *T. coccinea* needs to be further explored, for example, using a distinct polar extract of methanol.

It is also important to highlight the occurrence of a seasonal upwelling in the Southeastern Brazil, with high frequency and intensity between spring and summertime, specifically in Arraial do Cabo city (Calado et al. 2008; Castelao and Barth 2006). Studies with *T. coccinea* from Arraial do Cabo Bay showed a control interaction in the expansion and survival of colonies and recruits related to this phenomenon, with higher densities in areas with higher water temperatures (> 20 °C), and their absence in areas with cold upwelling waters (Batista et al. 2017). In addition to a warmer environment, the occurrence of *Tubastraea* species is also linked to lower salinity and high water transparency (Santos et al. 2019). Changes in those abiotic factors can occur on different time scales (Becerro et al. 1997; Duckworth and Battershill 2001; Turon et al. 1998). The production of secondary metabolites could exhibit significant temporal trends (Sacristán-Soriano et al. 2012). In this sense, this phenomenon has possibly played a significant role in the discrimination of samples since the production of chemicals differed significantly between seasons. In addition, coastal upwellings create a nutrient-enriched environment (Coelho-Souza et al. 2012), potentially contributing to the pronounced variation in metabolic content observed in *T. coccinea* colonies in spring.

Despite the sterol and fatty acids predominance in the three studied seasons (fall, winter and spring), the four populations of *T. coccinea*
exhibited several other substances, such as hydrocarbon, alcohols, aldehydes, and nitrogen compounds. All these chemicals have been already documented in coral species worldwide (e.g. Yamashiro et al. 1999; Harper et al. 2001; Changyun et al. 2008). It is also known that esters, sterols, and other lipids, including hydrocarbons, are constituents of coral mucus (Benson and Muscatine 1974; Nakajima et al. 2009). Studies carried out with Tubastrea sp. from Vietnam, for example, evidenced 32.2% phospholipids, 10.6% sterols, 1.7% fatty acids, 9% triacylglycerols, 3.1% monoalkyl diacylglycerol, and 40.3% fatty acid esters (Imbs 2013). Tubastrea coccinea and T. tagusensis from the Itacoatiba Island, southeastern Brazil, also showed a high abundance of the same metabolite class (Lages et al. 2010b).

Sterols are known in various marine invertebrates (Kanazawa 2001; Carreön-Palau et al. 2020). They are obtained by de novo synthesis or dietary sources and play essential roles in many biological processes (Kanazawa 2001). In T. coccinea colonies, sterols were the main and most frequent metabolites in fall, winter and spring samples. They were also found as major compounds in specimens of T. coccinea and T. tagusensis from other Brazilian coast regions (Lages et al. 2010b). Among the 15 species of corals analyzed from Oknawa, Japan, 11 of them had sterols, including the predominance of cholesterol in Tubastrea sp. (Yamashiro et al. 1999). In soft corals and gorgonians, sterols have multiple ecological roles, such as antifouling (e.g. Tomono et al. 1999) and defense against predators (Epifanio et al. 2007). These compounds might also be correlated with T. coccinea defensive system.

In general, hard exoskeleton corals have higher lipid contents than other cnidarians. Tubastrea sp. and the studied colonies of T. coccinea exhibited several lipid types of possible structural importance for the coral skeleton (Joseph 1979). The large amount of energy used to build up the coral skeleton would explain the increased lipid production (Imbs 2013). The lipid content depends on the light regime (Saunders et al. 2005) and is usually higher in summer due to the great intensity of solar energy. Seasonal variations in lipid contents might be associated with changes in metabolic rate due the seasonality of the sea surface temperature (Oku et al. 2003). Although the distribution of T. coccinea is associated with the predominant currents in this region, areas with higher temperatures showed the highest densities of colonies and recruits (Batista et al. 2017), and favored higher lipid production. In the coral Stylophora subseriata, higher lipid content was related to stress conditions (Seemann et al. 2012). However, in T. coccinea, this does not seem to be the reason for a high lipid abundance since it is well adapted to the prevailing environmental conditions in Arraial do Cabo Bay (Batista et al. 2017). Previous studies have suggested that fatty acids play a role as allelochemicals in aquatic organisms (e.g. Suzuki et al. 1996; Chiang et al. 2004).
Studies conducted with the stony coral *Acropora millepora* within an area of highly dynamic environmental conditions showed seasonal variation in its lipid profile. The total lipid content showed a clear cyclical pattern in which temperature was one of the main influencing variables (Conlan et al. 2020). In contrast, our study evaluated the colonies of the invasive coral *T. coccinea* in an undisturbed sheltered area (see Batista et al. 2017). We found a clear pattern of similarity in the profiles of secondary metabolites between populations at three times of the year (spring, fall and winter). Thus, based on a previous study (Batista et al. 2017), we can assume that the qualitative and quantitative chemical changes found could reflect the local environmental dynamics to which this coral is well adapted. Colonies of *T. coccinea* are circumscribed to the Arraial do Cabo Bay, where sea surface temperature varied between 12.5 and 29.0 °C for three years (Batista et al. 2017). This previous study showed that only *T. coccinea* colonies from Pedra Vermelha would eventually be subjected to temperatures below 15 °C. However, our n-MDS and PCA results indicated that the *T. coccinea* colonies from Pedra Vermelha did not differ significantly from Saco dos Cardeiros – SC, Ilha dos Porcos – IP, and Enseada do Anequim – EA. Two groups were discriminated against based on the chemical composition of the populations, one corresponding to samples close to the open ocean (PV and EA) and the other to warmer and more sheltered areas (IP and SC), although these differences were not significant. These results reveal a broad resistance of *T. coccinea* to environmental changes and supports its successful establishment and chemical adaptability in a relatively stable area, such as the one studied herein. In fact, *T. coccinea* is one of few coral species that did not suffer mortality under an intense warm water system (Robinson 1985).

Furthermore, lipids are drastically reduced in some soft coral species after spawning, indicating an overall energy demand for reproduction (Viladrich et al. 2016). As previously reported for the Great Barrier Reef in Australia (Ayre and Resing 1986) and the Eastern Equatorial Pacific (Glynn et al. 2008), *T. coccinea* can reproduce both asexually and sexually and this pattern is also observed on the Brazilian coast, with at least two periods of sexual reproduction per year and continuous larval production (De Paula et al. 2014). The lack of a pattern in the metabolic profile throughout the year could also be related to changes in lipid content. This content possibly fluctuates according to the spawning period, which has been observed more often in *Tubastrea* spp. than in other coral species (e.g. Conlan et al. 2020). Therefore, PCA results were discriminated by the unanimous contribution of lipids belonging to different chemical classes.

Biological, geographical, and physical factors influence the genetic connectivity between marine populations (Timm et al. 2017). The circumscribed distribution of *T. coccinea* to the Arraial do Cabo Bay is an excellent opportunity to carry out a small-scale connectivity study between

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Secondary metabolites variability in *Tubastrea coccinea* populations of this coral. Furthermore, it helps understand the qualitative and quantitative variability in the secondary metabolites in *T. coccinea* observed in the present study and the changes associated with environmental conditions.

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**Authors’ contribution**

Research conceptualization: ARS, NN, JASO, RCP; sample design and methodology: ARS; investigation and data collection: JASO, NN, ARS; data analysis and interpretation: ARS, JASO, NN, RCP; funding provision: ARS, RCP; roles/writing – original draft: ARS, JASO, NN, RCP; writing – review and editing: ARS, JASO, NN, RCP.

**References**


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Supplementary material

The following supplementary material is available for this article:

Figure S1. Chromatograms of the T. coccinea crude extracts collected in autumn season.

Figure S2. Chromatograms of the T. coccinea crude extracts collected in winter season.

Figure S3. Chromatograms of the T. coccinea crude extracts collected in spring season.

Table S1. Compounds annotated by GC/MS analysis of the T. coccinea extracts.