ARTHUR DE ALBUQUERQUE TENÓRIO

Shifts between autotrophy and heterotrophy in the reef-building coral *Mussismilia hispida*: an approach using fatty acid trophic markers

Thesis presented at the Oceanographic Institute of the University of São Paulo, as part of the requirements for obtaining the title of Master in Sciences, Oceanography Program, concentration area of Biological Oceanography.

Supervisor: Prof. Dr. Paulo Y. G. Sumida.

São Paulo

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SUMMARY

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RESUMO

Os recifes de coral estão entre os ambientes marinhos mais produtivos e ricos em biodiversidade. Esta biodiversidade está em parte associada a complexas estruturas formadas por corais escleractíneos. Apesar da importância ecológica, social e econômica dos recifes de corais, eles são expostos a várias ameaças relacionadas às atividades humanas. Dentre os impactos antrópicos em recifes, o branqueamento, ou perda de zooxantelas, é o mais notável e é diretamente relacionado à mortalidade dos corais. Por possuírem uma associação simbiótica com essas zooxantelas, alguns corais escleractíneos são considerados mixotróficos, caracterizados por modos de alimentação autotrófico (através de simbiose com o dinoflagelado *Symbiodinium*) e heterotrófico (predação sobre zooplâncton). Alguns estudos comprovam que corais com maior capacidade de alimentação heterotrófica são mais resistentes ao branqueamento e, consequentemente, às alterações climáticas. A fim de analisar se o coral escleractíneo *Mussismilia hispida*, é capaz de alternar seu modo nutricional entre predominante autotrófico e predominante heterotróficos, dezoito colônias foram amostradas ao longo de um ano. Marcadores Tróficos de Ácidos Graxos (FATM, na sigla em inglês) foram utilizados para determinar a fonte nutricional de carbono em tecido de corais. A concentração de células de *Symbiodinium* e a temperatura local também foram avaliadas. Branqueamento foi observado nos meses mais quentes do ano, quando a concentração de *Symbiodinium* diminuiu, voltando a aumentar nos meses mais frios. O marcador para dieta heterotrófica CGA (C20: 1ω9) foi encontrado em amostras de zooplâncton de toda a área de estudo. Em laboratório, colônias sem acesso a zooplâncton apresentaram perda significativa deste marcador após 10 dias. Amostras de colônias naturalmente branqueadas não apresentaram nenhum vestígio dos marcadores de autotrofia SDA (18: 4ω3) e DPA (22: 5ω3), mas continham tanto CGA e DHA (22: 6ω3). Isso confirmou que SDA e DPA são marcadores autotróficos viáveis e CGA é um marcador de heterotrofia. FATM relacionados com autotrofia apresentaram padrão semelhante ao observado para as concentrações de *Symbiodinium* e foram positivamente correlacionados com a densidade numérica de simbiontes e negativamente com a temperatura. Para explorar os dados de concentração dos FATM, o Índice Trófico de Corais foi desenvolvido para exibir as alternâncias entre modos nutricionais. *Mussismilia hispida* de fato alterna entre predominância de modo nutritivo ao longo do ano, sendo mais heterotrófica em períodos mais quentes e em condições climáticas adversas, porem na maior parte do ano é predominantemente autotrófica. A validação dos ácidos graxos marcadores tróficos específicos como referência para autotrofia e heterotrofia em corais abre perspectivas para novos estudos em ecologia trófica bêntica em recifes de coral. Este trabalho também inclui o primeiro monitoramento de um ano do comportamento alimentar em um coral hermatípico no Atlântico Sul e o acompanhamento de um evento de branqueamento.

PALAVRAS-CHAVE: Autotrofia, Branqueamento, Coral, FATM, Heterotrofia, Mixotrofia, *Symbiodinium*.

ABSTRACT

Coral reefs are among the most productive and biodiverse marine environments. This remarkable biodiversity is partly associated to the complex structures formed by scleractinian corals. Despite the ecological, social and economic importance of coral reefs, they are constantly exposed to several threats mainly related to human activities. Climate changes are one of the most notable impacts of human activity related to coral mortality, mainly due to coral bleaching. Some scleractinian corals are proved to be mixotrophs, displaying both autotrophic (through *Symbiodinium*) and heterotrophic (predation on zooplankton) nutrition modes. Many studies emphasize that corals with greater capability of heterotrophic feeding are more resilient to bleaching and consequently to climate change. In order to analyze whether the endemic scleractinian coral *Mussismilia hispida* is capable of shifting from predominant autotrophic and predominant heterotrophic in Ubatuba-SP, 18 colonies were sampled monthly for 12 months. The Fatty Acid Trophic Markers (FATM) approach was used to determine the source of carbon on coral tissues. *Symbiodinium* cell density and local seawater temperature were also assessed. A mild bleaching was observed showing a decrease in *Symbiodinium* numerical density during warmer months, but increasing in colder months. Reference samples validated the relation between all selected FATM and its corresponding nutritional mode. The heterotrophic feeding marker CGA (C20:1ω9) was found in zooplankton samples collected throughout the study area. Laboratory starved colonies (no access to zooplankton) lost any trace of this marker after 10. Samples from naturally bleached colonies presented no traces of the autotrophic feeding markers SDA (18:4ω3) and DPA (22:5ω3), but contained both CGA (C20:1ω9) and DHA (22:6ω3). These results confirmed that the FATM analyzed where reliable trophic markers. Autotrophic FATM presented a pattern similar to that observed for *Symbiodinium* concentration in *M. hispida tissues* and were positively correlated with the symbiont and negatively with temperature. The Coral Trophic Index showed that *M. hispida* undergoes shifts in nutritional modes along the year, being more heterotrophic in adverse conditions. The validation of specific FATM as proxies for autotrophic and heterotrophic feeding in corals opens new perspectives for further studies in benthic trophic ecology in coral reefs. This work also presents the first yearlong monitoring of the feeding behavior in a hermatypic coral in the South Atlantic and the monitoring of a mild bleaching event.

KEYWORDS: Autotrophy, Bleaching, Coral, FATM, Heterotrophy, Mixotrophy, *Symbiodinium*.

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INTRODUCTION

Coral reefs are among the most productive and biodiverse marine environments (Connell, 1978; Schnell, 2004; Sheppard *et al*., 2009). Even though they are mostly found under oligotrophic conditions, coral reefs are highly productive ecosystems because of the photosynthesis performed by symbiotic dinoflagellates and macroalgae (Falter *et al.,* 2011). Despite occupying an area less than 300,000 km^2 corresponding to 1% of the ocean surface area (Sheppard *et al*., 2009), this ecosystem provides habitat for more than 25% of the known marine species, including one third of marine fish species, 5,000 mollusk species, 800 hard corals, and a large number of echinoderms, polychaetes, sponges and crustaceans (UNEP, 2003, Schnell, 2004; Lalli and Parsons, 2006 Sorokin, 2013). With the exception of Onychophora, all metazoan phyla are found in coral reefs making this the most diverse environment on Earth at higher taxonomic levels (Glynn and Enochs, 2011).

The high biodiversity of coral reefs is of relevant economical importance, since approximately 10 million people depend directly on them (Salvat, 1992; Wilkinson, 2008). The main commercial activities related to coral reefs include tourism (diving centers, hotels and restaurants – see Moberg and Folke, 1999), fisheries (fishes such as parrotfishes and groupers are important examples of coral reef species harvested for human consumption – see Bellwood and Wainwright, 2002), production of pharmaceuticals and medication (Cinel *et al.,* 2000; Alves de Lima *et al*., 2013) and the marine aquarium trade (more than 1,000 ornamental fish species are commercially targeted in an economic activity that is worth more than U$ 15 billion – see Wabnitz *et al*., 2003; Olivotto *et al*., 2006). These activities generate roughly U$ 797 billion every year (Cesar *et al.,* 2003).

Despite the ecological, social and economic importance of corals reefs, they are constantly exposed to several threats mainly related to human activities (Roberts *et al*., 2002; Burke *et al*., 2011). These include marine pollution, overfishing, habitat destruction and climate change associated with carbon dioxide emissions that alter water temperature and carbonate chemistry (Calado, 2006; Hoegh-Guldberg *et al*., 2007). These have destroyed approximately 20% of coral reefs worldwide, while another

35% are seriously threatened (Wilkinson, 2008). In some cases, anthropogenic impacts to coral reefs have exceeded their regenerative capacity, causing dramatic shifts in species composition and severe biodiversity reduction, which directly led to economic loss (Bellwood *et al*., 2004).

The remarkable biodiversity found in coral reefs is partly associated to the complex calcium carbonate structure formed by scleractinian corals (Cnidaria: Anthozoa). These are the true reef-building organisms, also called hermatypic, which provide structural habitat for numerous other species (Krueger *et al*., 2015). Surface irregularities, crevices and tunnels in the reef limestone form a variety of microhabitats and the erosion of the main reef structure creates areas of rubble and sand around it (Hallock, 1997). Most hermatypic corals live in clear, oligotrophic and shallow tropical waters (Sheppard *et al*., 2009). The rate at which hermatypic corals deposit calcium carbonate depends on the species, but some of the branching species (*e.g*., *Acropora* spp.) can grow approximately 10 cm per year (Ruppert *et al*., 2004), thus making coral reef damage recovery a slow and fragile process.

Some species of hermatypic corals live in a mutualistic association with unicellular photosynthetic dinoflagellates of the genus *Symbiodinium*, also known as zooxanthellae (Stat *et al*., 2006; Venn *et al.,* 2008). The coral host provides shelter, nitrogen, phosphorus and carbon dioxide to the symbiotic dinoflagellate, which in return translocates up to 95% of its photosynthetically-fixed carbon to the host (Falkowski *et al.,* 1984; Muscatine and Weis, 2013). *Symbiodinium* dinoflagellates may contribute up to 100% of the coral daily metabolic needs (Papina *et al.,* 2003; Grottoli *et al.*, 2006; Hoegh-Guldberg *et al.,* 2007). For many years reef-building corals were considered mainly dependent on the photoautotrophy performed by *Symbiodinium* (Muscatine and Porter, 1977). However, photosynthetic products may be deficient in nitrogen and phosphorus (Crossland, 1987; Wild et al., 2004) and essential nutrients for growth and reproduction may need to be acquired through a different feeding source (Ferrier-Pagès *et al*., 2003).

Numerous studies have confirmed that most coral species can in fact be active heterotrophs and prey on plankton organisms (Sebens *et al*., 1996; Grottoli, 2002; Houlbrèque *et al.*, 2004; Palardy *et al*., 2005, 2006; Houlbrèque and Ferrier-Pagès,

2009). Heterotrophic input is necessary for maximal coral growth and it can contribute up to 66% of the fixed carbon found in both coral tissue and skeleton (Grottoli and Wellington, 1999; Houlbrèque *et al*., 2003; Palardy *et al.,* 2005). The ability to feed as both autotrophs and heterotrophs renders corals the status of mixotrophs.

Mixotrophy supplies corals with different compounds, including a wide range of lipid compounds (Crossland *et al.,* 1980; Grottoli *et al*., 2006), which play an essential role in coral nutrition and metabolism. Among these, fatty acids (FA) are important since they may be used in ecological studies as trophic markers (*i.e*., fatty acid trophic makers - FATM) (Sargent, *et al*., 1990; Volkman, 1999; Dalsgaard *et al*., 2003). Animals, differently from many phototrophs, cannot produce FA with unsaturated bonds beyond a determinate carbon position (position Δ^9; Dewick, 1997). Moreover, animals are incapable of synthesis *de novo* for most FA (Dalsgaard *et al.,* 2003) and these are produced through different cellular pathways. In animals, this occurs in the phospholipids of the endoplasmic reticulum, whereas in most autotrophs FA are synthesized in the thylakoid membranes of chloroplasts (Papina *et al*., 2003). The only manner in which animals can obtain polyunsaturated fatty acids is by consuming organisms that produce them (such as phytoplankton) or organisms that have consumed a producer (Persson and Vrede, 2006). Specific FA obtained in such ways may then serve as food-source markers (Sargent *et al.*, 1990; Dalsgaard *et al*., 2003).

Many studies used the approach of FATM to evaluate carbon source in scleractinian corals (Wilson *et al*., 2001; Treignier *et al.*, 2008; Dodds *et al.,* 2009; Figueiredo *et al*., 2012). It is known that specific FATM in coral tissues may be traced back to the photosynthetic contribution of *Symbiodinium*, such as 18:4ω3, 22:5ω3 and 22:6ω3 (Papina *et al*., 2003; Treignier *et al.,* 2008). Other FA may be specific to heterotrophy, such as those produced by crustacean zooplankton (*e.g.*, C20:1ω9 and C22:1ω11; Dalsgaard *et al*., 2003), which are relevant prey items for hard corals.

However, both autotrophy and heterotrophy may be regulated by abiotic factors. Increased seawater temperature is associated with declines in photosynthetically-driven carbon fixation rates (Clark and Jensen, 1982) and with decrease in calcification rates, tissue biomass and FA reserves (Grottoli *et al.,* 2006; Rodrigues and Grottoli, 2006, 2007; Cantin *et al.,* 2010; Levas *et al.,* 2013). High temperatures and excessive light

exposure are the main causes of coral bleaching, which is the paling or whitening of corals as a result of the loss of *Symbiodinium* or a decrease in its pigment content (Douglas, 2003). In addition, *ex situ* experiments show that corals upregulate heterotrophic feeding when photosynthesis is suppressed either due to decreased water clarity and deprivation of sun light (Anthony and Fabricius, 2000; Hoogenboom *et al., 2010*) or when symbionts are lost from coral tissue due to bleaching (Grottoli *et al*., 2006). The local zooplankton community composition may as well affect heterotrophic intake because different species of coral prey on certain zooplankton size ranges (Palardy *et al*., 2006). However, many studies have demonstrated that not all species are capable of using heterotrophy to compensate for reduced photosynthesis (Anthony and Fabricius, 2000; Grottolli *et al.*, 2006). Despite all of this information, no study has monitored *in situ* if hermatypic corals shift between feeding modes and what may regulate these shifts.

The present work aimed to determine if the scleractinian coral *Mussismilia hispida* is both autotrophic and heterotrophic and to investigate the influence of abiotic factors on the relative intensity of those trophic modes. This work is based on the following hypotheses: i) *Mussismilia hispida* shifts its feeding mode between predominantly autotrophic and predominantly heterotrophic; and ii) *Mussismilia hispida* is predominantly heterotrophic throughout the year. In order to answer these questions, we monitored the concentration of specific FATM related to both autotrophy and heterotrophy in *M. hispida* colonies for a year at Ubatuba (São Paulo state, Brazil), while also taking continuous measurements of seawater temperature.

Mussismilia hispida is an endemic and hermatypic species of the Brazilian coast and Ubatuba is almost at its distribution limit to the south being the only hermatypic coral species found in the São Paulo state. Understanding how this species feeds and thrives at such higher latitudes may be important not only for coral reef trophic ecology, but also may contribute to the current knowledge of possible habitat expansion of more resistant species and adaptation facing climate change.

MATERIALS & METHODS

Study area and experimental design

Sampling took place in the coastal region of the town of Ubatuba (São Paulo state), Brazil (23°30'01.9"S 45°07'07.9"W). This area is under the influence of the relatively warm and low salinity Coastal Water (CW) and seasonal summer intrusions of the South Atlantic Central Water (SACW), with lower temperatures and higher salinity (Castro Filho *et al*., 1987). Sea surface temperature in the area ranges from 14.6 to 27.9°C and salinity from 31.7 to 35.8 ppt (Castro, 1995). Low intensity waves come from the East and stronger waves come from South and Southwest (Castro Filho *et al*., 1987). Fine sand, silt and clay predominate, while coarser material is found in enclosed areas (Mahiques, 1995)

Six experimental sites were selected in Flamengo and Fortaleza Bays and Anchieta Island (Fig. 1). In each site, three colonies of the Brazilian endemic scleractinian coral *Mussismilia hispida* (Fig. 2) were tagged and monthly sampled. In order to have replicated individuals, colonies were chosen in areas with similar conditions, *i.e.*, similar position/orientation on the substrate, depth of 3 m ± 0.75 m, direct exposure to sunlight and near absence of competing organisms such as turf and frondose algae and sessile invertebrates like barnacles and mussels. All colonies were sampled nearly monthly for a year (Feb/2015 – Jan/2016), with intervals varying from 20 – 40 days, depending on climate and hydrodynamic conditions. Approximately one polyp from each colony was collected monthly with hammer and chisel. Immediately after removal, polyps were placed in tagged plastic bags with seawater and refrigerated on ice.

Seawater temperature was continuously recorded at each site using HOBO Onset®data loggers (accuracy of ± 0.53°C; resolution of 0.14°C at 25°C) (Fig. 3). In every sampling event data loggers were cleaned and the recorded information was retrieved. All temperature data are presented as a continuous measurement for each site. The local mean temperature was calculated individually for each site using the whole data set, specific to each site. Temperature means used for comparisons with *Symbiodinium*

concentration and fatty acid trophic markers (FATM) were calculated using all of the temperature data recorded in between each sampling period.

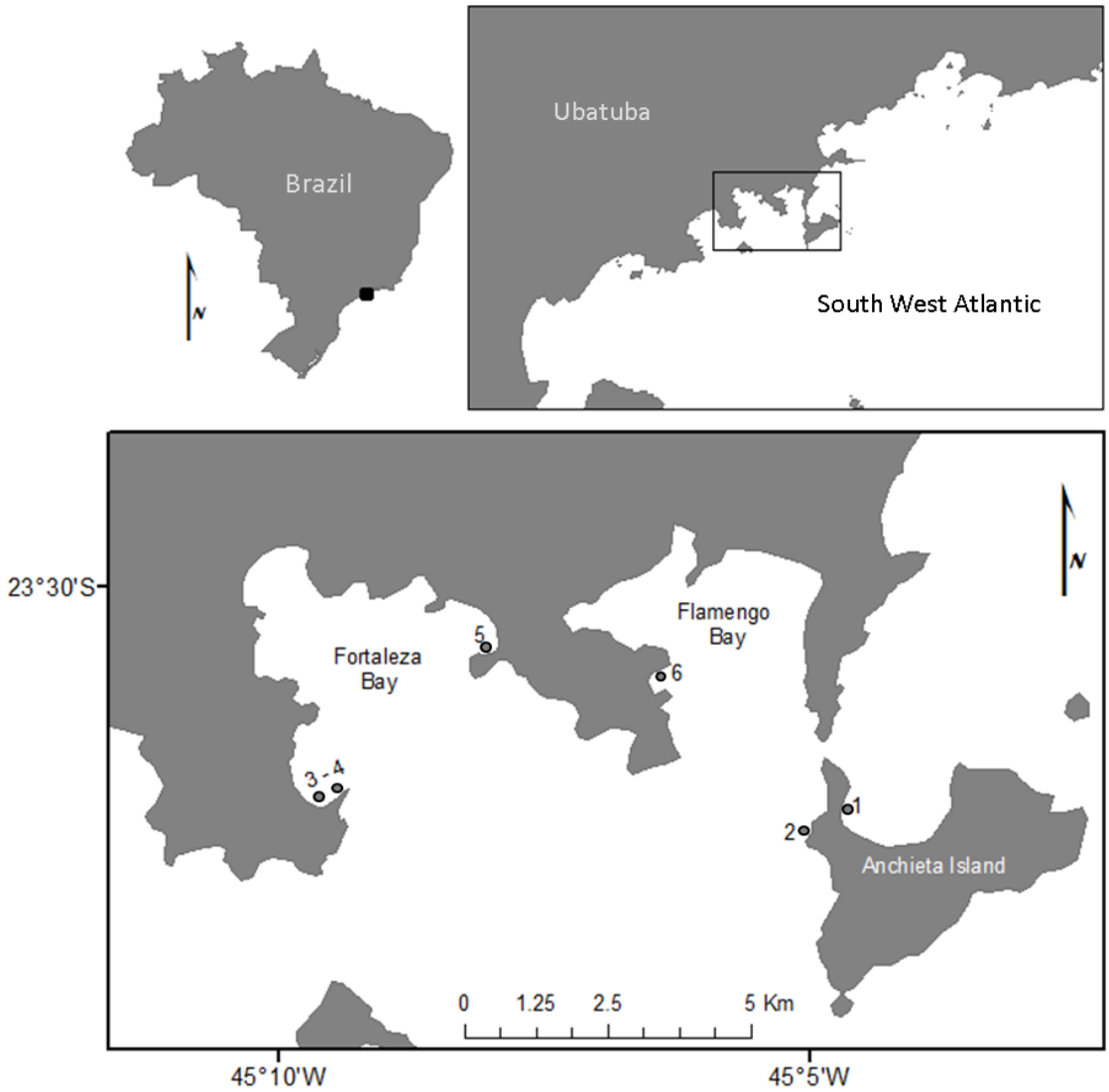

Figure 1. Sampling locations of *Mussismilia hispida* colonies along the coast of Ubatuba. 1: Anchieta I 2: Anchieta II 3: Fortaleza I 4: Fortaleza II 5: Lázaro 6: Praia do Flamengo. *Mussismilia hispida* colonies were tagged and tissues were sampled monthly in each location. Samples were analyzed for *Symbiodinium* concentration and fatty acids related to autotrophic and heterotrophic feeding. Illustration by Romina V. Barbosa.

Table 1. Geographic coordinates for each site of *Mussismilia hispid*a sampling.

	Location	Coordinates
1	Anchieta I	S 23°32'02.5404"; W 45°04'40.8144"
2	Anchieta II	S 23°32'39.2820"; W 45°04'46.3800"
3	Fortaleza I	S 23°31'52.2372"; W 45°09'44.0748"
4	Fortaleza II	S 23°31'46.4232"; W 45°09'31.4064"
5	Lázaro	S 23°30'30.2364"; W 45°08'11.0904"
6	Praia do Flamengo	S 23°30'37.6164"; W 45°06'29.5200"

Ocean and climate data were also obtained from CPTEC/INPE (Centro De Previsão de Tempo e Estudos Climáticos/Instituto Nacional de Pesquisas Espaciais) and monthly-accumulated rainfall was obtained from NAP-Oceano Sustentável (Núcleo de apoio à pesquisa oceano sustentável). Percentage of cloud coverage data and mean wave height and direction were compiled for each week and month of 2015 and for January 2016. For the periods in between samplings, accumulated rain and percentage cloud coverage were calculated as well.

Sampling was not performed in July due to rough weather, as waves were frequently higher than 2.0 m and hindering sampling conditions. Tagged colonies from Anchieta II were not sampled in July, September and October, as they were not found due to poor water visibility and weather conditions.

Figure 2. *Mussismilia hispida* colonies found in Ubatuba. Three colonies were selected at each location in similar conditions, including orientation in the substrate, depth (3 m ± 0.75 m), and absence of fouling and competitors such as frondose macroalgae.

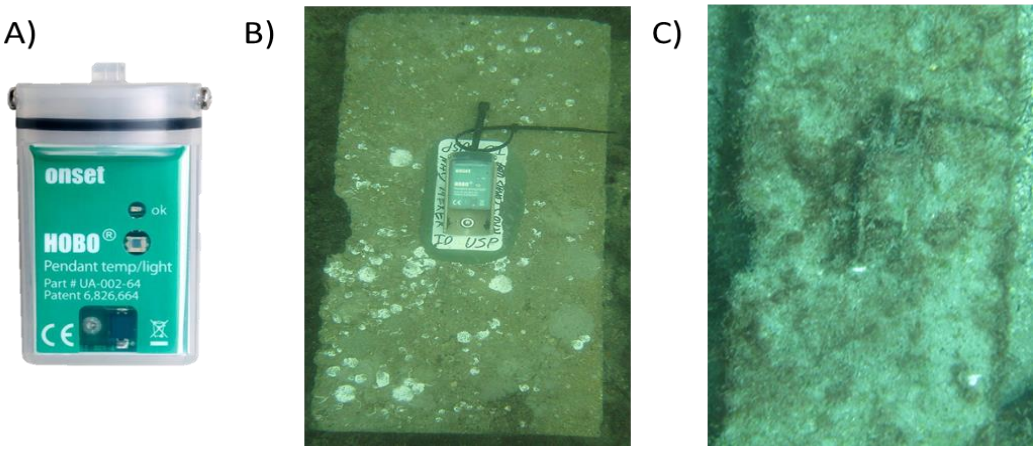

Figure 3. (A) HOBO data logger (Onset®, accuracy of ± 0.53°C; resolution of 0.14°C at 25°C) for the continuous recording of temperature; (B) Structure built to fix loggers on the substrate next to *Mussismilia hispida* colonies; (C) Fouling on data logger and structure after 35 days at sea.

Coral tissue sample processing

Major skeleton fragments were removed with pliers and tweezers. Each sample was then divided in two aliquots of approximately 0.1 g each. The aliquots were used for (i) *Symbiodinium* cell count and (ii) fatty acid extraction for gas chromatography analysis.

Symbiodinium cell count

Skeleton fragments were removed from soft tissue through sample homogenization and decantation. Excess water from the tissues was removed by briefly stirring the sample on a dry petri dish followed by weighing. Samples were then transferred to 2-mL centrifuge tubes with 1 mL of distilled water. The contents were grinded with a pestle, homogenized for 5 minutes and agitated for 3 minutes. The resulting solution was filtered through a 100-µm mesh in order to remove unwanted debris, and then washed with 4 ml of distilled water. The final filtered solution had a volume of 5 ml. *Symbiodinium* cells were counted in a Nageotte counting chamber (Fig. 4). The number of *Symbiodinium* cells per mg of coral soft tissue was obtained according to equation (1).

$$(1)\ Symb/mg_{cst} = \frac{(1{,}25CC \times 1000NL) \times 5}{SW}$$

Where:

cst - Coral soft tissue;

CC - Counted *Symbiodinium* cells;

NL - Counted Nageotte chamber lines;

SW - Coral soft tissue weight.

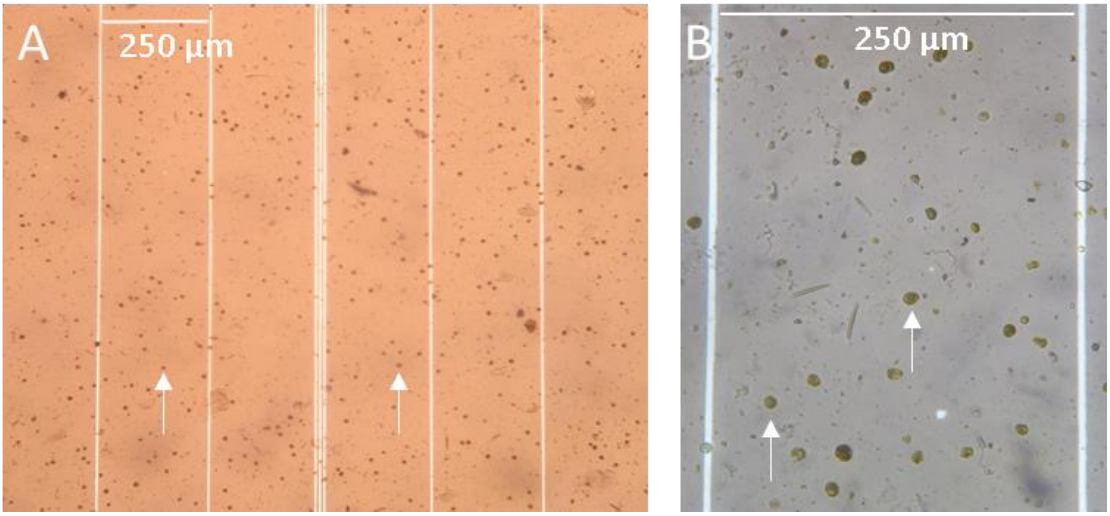

Figure 4. *Symbiodinium* cells (arrows) from the coral *Mussismilia hispida* in the Nageotte counting chamber. (A) 10 x magnification; (B) 40 x magnification.

Fatty acid extraction

Lipids were extracted from samples using a method adapted from Bligh & Dyer (1959). The soft tissue (skeleton-free) was weighed and added to a glass tube. One milliliter of a solution of 100% methanol and 100% dichloromethane (1 : 1) was added to each tube and agitated for one minute, followed by the addition of 100 µL of internal standard (tricosanoic acid, C23:0). Samples were then esterified with 5 mL of a 1 : 0.02 solution of methanol and sulfuric acid. Subsequently, 2 mL of 100% hexane was added and agitated. Condensers were attached to the tubes and placed in a 50°C bath for 30 minutes. The tubes were cooled to room temperature and phases separated with a 5 mL saturated solution of NaCl. The top phase was retrieved (2 mL) for GC analysis. When samples weighed less than 50 mg, concentration to 1 mL was performed by evaporation with pure (99.999%) Nitrogen gas.

Gas chromatography

Esterified samples were analyzed in a 6890 Agilent gas chromatograph coupled with a flame ionization detector (GC-FID). The column used was the Agilent J & W (50 m long, 0.35 mm internal diameter and 0.17 mm thick with a 5% phenylmethylsiloxane film). Two microliters of the sample were injected and carried by ultra-pure hydrogen and nitrogen gases. The oven ramp temperature started at 50 °C, and kept constant for

2 minutes, then it increased at the rate of 9°C min^{-1}, remaining constant until it reached 300°C and maintained for 10 minutes.

The concentrations of four fatty acids were investigated: stearidonic acid (SDA, 18:4ω3), docosapentaenoic acid (DPA, 22:5ω3), docosahexaenoic acid (DHA, 22:6ω3) and cis-gondoic acid (CGA, C20:1ω9). The first three are markers for autotrophy (translocated from *Symbiodinium* to the host – see Papina *et al*., 2003) whereas the last one is for heterotrophy (CGA is found in copepods – see Dalsgaard *et al*., 2003). The identification of methyl esters of fatty acids was done by comparison of the retention times of the target fatty acids with a reference analytical standard (47033 PUFA No. 1 Marine Source - Sigma-Aldrich Co.®), based on the calibration curves fitted with at least 5 different concentrations.

Fatty acid trophic markers reference samples

Three different reference samples were produced in order to confirm if the investigated FATM were adequate. The first group of reference samples was the monthly night-time collection of zooplankton on each site to confirm the presence of CGA in zooplankton organisms. This is a marker specific to crustaceans (Dalsgaard *et al*., 2003) and was selected as a proxy for heterotrophic feeding. Zooplankton oblique tows were made with a 300-µm mesh conical net for 5 minutes at 3 knots.

The second group of reference samples was produced through an experiment that intended to verify if CGA found in the host tissue was indeed related to heterotrophic feeding. For that purpose, four *Mussismilia hispida* colonies (Fig. 5) were maintained for 10 days in a tank with filtered seawater and no zooplankton at a temperature of 25°C ± 1.8, salinity of 34 and with indirect sunlight. Approximately 70% of the water was changed daily and samples were taken every two days to monitor the concentration of CGA in the host tissue and to observe the concentration of *Symbiodinium* cells concentration. From hereafter we call these starving colonies. Day 0 were sampled prior to colonies removal from the environment and it was considered

as a stress period due to removal disturbances and adaptation to the experiment system.

The last analysis had the purpose of verifying if the three autotrophy FATM, *i.e.*, SDA, DPA and DHA, were specific to *Symbiodinium* as previously reported (Papina *et al*., 2003). For that, we collected bleached colonies in which there would be little or no *Symbiodinium* (Fig. 6) and most likely a very small or undetectable amount of these FATM.

Figure 5. Experimental set up to assess the decrease of heterotrophic FATM (CGA – cis gondoic acid) contents in *Mussismilia hispida* tissue under starving conditions (i.e., absence of zooplankton). Left: Recirculated aquaria built for keeping the colonies. Right: Close up of the experimental colonies in the beginning of the experiment.

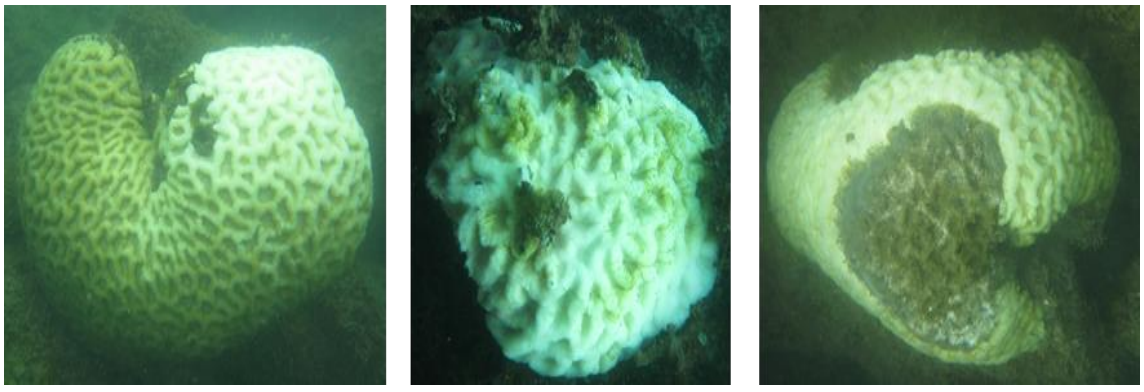

Figure 6. Bleached *Mussismilia hispida* colonies sampled *in situ* to assess the autotrophic FATM (SDA, DPA and DHA) content and *Symbiodinium* cells concentration in the coral tissue.

Statistical analyses

Mean temperature differences among sampled months were tested using a one-way Analysis of Variance (ANOVA), using sites as replicates (one temperature sensor per site, n=6). Mean temperature differences among sites was tested also with a one-way ANOVA, using sampled months as replicates (n=11; in October 2015 at Lázaro, the sensor was lost).

For the starving experiment, each FATM (DHA, SDA, DPA and CGA) was tested for differences among sampling times using a one-way ANOVA. Differences between individual sampling times for each FATM were tested for significance with a pairwise *post-hoc* Tukey test.

Symbiodinium cell concentrations were tested for differences among sites and sampling periods using a 2-way nested ANOVA, with the sampled months nested within each site. If significant difference was found, each pair was compared for significant differences with a *post-hoc* Tukey test.

Significant relationships between pairs of variables, such as *Symbiodinium* concentration and mean temperature, FATM and mean temperature, and between the specific FATM (DHA, SDA, DPA and CGA) to *Symbiodinium* concentration was assessed using Pearson's correlation test.

Statistical analyses were performed using the statistical packages JMP (version 8). Biological data (*Symbiodinium* cell concentration and FATM concentrations) were log-transformed [log(x+1)] to guarantee homoscedasticity and normalization requirements for parametric tests (ANOVA and Tukey). All the figures were produced with Grapher 8 and Microsoft Excel software.

Coral Trophic Index

In order to answer the proposed hypotheses, three equations (2-4) were created using the results of the concentrations of fatty acids for both autotrophy and heterotrophy. Equations (2) and (3) determine if the intensity of each trophic mode increased or decreased from one sampling month (t0) to another (t1). The difference between equations (2) and (3) was calculated to determine which of the trophic modes predominates in a colony at a specific moment. The resulting value was denominated Coral Trophic Index (CTI). A positive value reflects a predominantly autotrophic feeding behavior, whereas negative values indicate a predominantly heterotrophic behavior.

$$(2)\ \text{heteroFATMRatio} = \left[\left(\frac{Xt1heteroFATM}{Xt0heteroFATM}\right) - 1\right]$$

$$(3)\ \text{autoFATMRatio} = \left[\left(\frac{Xt1autoFATM}{Xt0autoFATM}\right) - 1\right]$$

$$(4)\ \text{CTI} = (\text{autoFATMRatio} - \text{heteroFATMRatio})$$

Where:

Xt1heteroFATM = [µg g^{-1}] of CGA of sample x at t1

Xt0heteroFATM = [µg g^{-1}] of CGA of sample x at t0

Xt1autoFATM = [µg g^{-1}] of autoFATM (SDA + DPA) of sample x at t1

Xt0autoFATM = [µg g^{-1}] of autoFATM (SDA + DPA) of sample x at t0

DISCUSSION

The present work aimed to determine whether the Brazilian endemic scleractinian coral *Mussismilia hispida* is capable of shifting between nutritional modes and to investigate if heterotrophy is the most predominant mode throughout the year. To answer the proposed hypotheses, specific fatty acid trophic markers (FATM) were used to identify the source of fatty acids (FA) present in *M. hispida* tissues at Ubatuba. The FATM approach is based on the premise that the selected markers cannot be selectively processed through food uptake or incorporation and are not synthetized by the targeted animal, instead they should be transferred from one trophic level to the next by incorporation or ingestion from the animal nutritional source (Napolitano *et al*., 1997; Dalsgaard *et al*., 2003; Iverson *et al*., 2004). Despite strong evidence that SDA, DPA and DHA are translocated from *Symbiodinium* to its coral host (Papina *et al*., 2003) and that CGA is a specific marker for crustacean zooplankton (Dalsgaard *et al*., 2003), we produced three reference samples in order to confirm that these markers were adequate indicators of autotrophic and heterotrophic carbon input for *M. hispida*.

Scleractinian corals of many different species have been observed to prey on zooplankton (Ferrier-Pagès *et al*., 2003; Sebens *et al*., 2003; Palardy *et al*., 2005). There is evidence that within the zooplankton, copepods are the dominant food source for corals (Dodds *et al*., 2009) and CGA has been reported as a FATM produced by calanoid copepods (Dalsgaard *et al*., 2003). These are the most abundant organisms found in zooplankton communities at Ubatuba (Lopes, 2003). The first group of reference samples demonstrated that CGA was found in all zooplankton samples collected at all locations throughout the year, thus showing that this marker is representative of the local community.

Under controlled laboratory conditions, *M. hispida* colonies were placed in closed and recirculated aquaria without the presence of zooplankton (*i.e*., in starving conditions), but with free access to sunlight. The concentration of CGA significantly decreased during this period and the FATM for autotrophy (SDA, DPA and DHA) did not; in fact, after the sixth day they increased. Therefore, coupled with the zooplankton

samples, these results confirm that CGA can be regarded as an effective trophic marker for heterotrophic feeding in *M. hispida*, reassuring the findings in Dalsgaard *et al.* (2003).

The last reference samples consisted of three highly-bleached colonies that were found and collected at Ubatuba. Both FATM SDA and DPA were absent and the heterotrophy marker (CGA) was found at much higher concentrations. This confirms that that SDA and DPA found in *M. hispida* are specific to *Symbiodinium*. The corals were likely compensating symbiont loss with prey capture (Grottoli *et al*., 2006). However, DHA was found in these bleached colonies. This is most likely related to the fact that DHA is not specific to dinoflagellates, but is also abundantly produced by many phytoplankton species (Volkman, 1999), and may have been present in zooplankton that had consumed diatoms and microalgae. Therefore, despite being produced by *Symbiodinium*, DHA is an ambiguous marker and was excluded from most of the analyses. Thus, the combination of SDA and DPA (defined as the autoFATM) is an adequate FATM for the autotrophic contribution from *Symbiodinium* to the coral host.

It is important to note that the concentration of SDA and DPA is not a proxy for the concentration of *Symbiodinium* in the coral tissue. Production rates of these fatty acids vary considerably (Titlyanov *et al.,* 2001; Zhukova and Titlyanov, 2002), especially due to cell size, shape, strain and associated host (Bishop and Kenrick, 1980; Titlyanov *et al.,* 2001). For instance, *Symbiodinium* undergoing photoacclimation typically produce higher concentrations of photosynthates (Anthony and Hoegh-Guldberg, 2003a, 2003b; Hoogenboom *et al.*, 2006). Furthermore, a very weak correlation was found between *Symbiodinium* concentration and the combined content of SDA and DPA ($r_{(186)} = 0.387$). Therefore, we consider an increase in SDA and DPA as an increase in their production by the *Symbiodinium* population inside the coral tissue.

Our first hypothesis was confirmed as the Coral Trophic Index (CTI) constantly oscillated between positive and negative values, showing that indeed there are shifts between predominantly autotrophic and predominantly heterotrophic feeding modes (Fig. 22). It is generally accepted that most of these shifts are related to turbid waters (Anthony, 2000; Fabricius and Dommisse, 2000), temperature changes, reduced photosynthesis and *Symbiodinium* concentration, as a compensatory strategy (Ferrier-Pagès *et al.*, 2003; Palardy *et al.*, 2005; Grottoli *et al.,* 2006; Houlbréque and Ferrier-

Pagés, 2009). While we expected that temperature would be the major factor driving theses shifts, as reported in (Rodrigues and Grottoli, 2007; Palardy *et al.,* 2007; Connolly *et al*., 2012; Ezzat *et al.,* 2016), we found no correlation between the CTI and temperature or temperature variations. However, these shifts do coincide with the sporadic adverse weather conditions reported. These conditions include strong rainfall from February to March, turbulent hydrodynamics and sediment resuspension from July to August and increased temperature and cloud cover from October to November (Table 2 and Fig. 7). All these events coincided with shifts from autotrophy to heterotrophy (Fig. 22). Intense weather has been linked with coral damaging (Crabbe *et al.*, 2008), while increased rainfall, severe terrestrial runoff and high wave energy have been reported to cause severe damage, bleaching and/or mortality of coral colonies (Connell, 1997; Connell *et al*., 1997; Mallela and Crabbe, 2009).

Our second hypothesis was rejected, as the CTI indicates that *M. hispida* was predominantly autotrophic, for 6 periods out of 10, which represent the greater part of the experiment. Heterotrophy was expected to predominate due to the environmental conditions found at Ubatuba, which is near the limit of the species distribution (*e.g*., low seawater temperature in the winter and autumn and high turbidity throughout the year). These are the main factors that lead the corals to upregulate heterotrophy and suppress photosynthesis (Anthony and Fabricius, 2000; Grottolli *et al*., 2006; Hoogenboom *et al*., 2010; Levas *et al*., 2013). Our results show that this species resorts to heterotrophy when adverse climate conditions seem to hinder photosynthetic production by *Symbiodinium*.

Interestingly, our data has shown that *M. hispida* colonies in Ubatuba go through a mild seasonal bleaching during the warmer months in the summer and recover during the remainder of the year when temperatures are lower (Fig. 10 and 11). The phenomenon of coral bleaching is defined by the loss of *Symbiodinium* cells, resulting in accessory pigment breakdown and subsequent tissue loss, leaving only the white skeleton (Iglesias-Prieto *et al*., 1992; Hoegh-Guldberg, 1999; Fitt *et al*., 2001). The main cause of bleaching is elevated seawater temperature (Fitt et al., 2001) and data collected at Ubatuba revealed a yearly oscillation of 15°C, which is significantly high for hermatypic corals. Another experiment did monitor seasonal variation in S*ymbiodinium*

concentrations in *M. hispida* and other two species of endemic corals at Picãozinho (Paraíba State, Northeast Brazil), but no bleaching was detected (Costa *et al.,* 2005).

Several parameters such as temperature, salinity, nutrients, light availability and hydrodynamics define the geographical limits for the occurrence of scleractinian corals (Smith and Buddmeiro 1992; Kleypas 1997; Kleypas *et al*., 1999). The distribution limit of *Mussismilia hispida* is the state of São Paulo (approx. 24° S) (Francini *et al.,* 2002), and at Ubatuba (S 23º 26' 02"; W 45º 04' 16") they are considered to be marginal to their distribution (Leão *et al*., 2003). *M. hispida* is the only reef-building representative of the order Scleractinia in the region, and it has been reported as capable of spawning in this area (Francini *et al*., 2002). The heterotrophic feeding by *M. hispida* may be an important adaptation to less than optimal conditions, as they may resort to this nutritional mode when photosynthesis is suppressed. It has been reported that, after prolonged bleaching events, corals without significant input of heterotrophic carbon such as *Porites* are expected to be more susceptible to mortality than corals such as *Montipora capitata*, which presents high rates of heterotrophic feeding (Grottoli *et al*., 2006). This fact suggests that *Mussismilia hispida* has an ecological advantage over other species due to its ability to feed on zooplankton and survive during prolonged periods of bleaching (Grottoli *et al*., 2006). Furthermore, it is feasible that due to its heterotrophic capacity, *M. hispida* has evolved and adapted to survive and reproduce under suboptimal conditions, expanding its distribution. These adaptations may prove crucial in view of current climate change projections, since this species may expand its distribution to areas previously occupied by less tolerant species. In the future, the disproportional presence of *M. hispida* could maybe used as a proxy for coral habitat degradation.

Our major findings show that *M. hispida* is able to frequently shift between predominantly autotrophic and predominantly heterotrophic, especially when adverse climate events occur. Furthermore, we have validated the use of specific fatty acid trophic markers as proxies for these feeding modes in corals. This opens perspectives for further studies of benthic trophic ecology in coral reefs. The contribution of this work also includes the first yearlong monitoring of the feeding behavior in a hermatypic coral in the South Atlantic, with the occurrence and monitoring of a mild bleaching event.

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