Journal of Experimental Marine Biology and Ecology 474 (2016) 133–141

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Nutrient acquisition strategies in mesophotic hard corals using compound specific stable isotope analysis of sterols

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ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form 18 October 2015
Accepted 19 October 2015
Available online 28 October 2015

Keywords:
Mesophotic reefs
Montastraea cavernosa
Agaricia species
Sterols
Phytosterols
Stable isotopes

ABSTRACT

This study assessed the nutrient acquisition strategies of two scleractinian corals, *Montastraea cavernosa* and *Agaricia* spp., collected from shallow (depths less than 20 m) and mesophotic (depths of 30–150 m) habitats. The composition of biomarker sterols, bulk stable carbon and nitrogen isotope values, and compound specific stable isotope analysis (CSIA) of the sterols were analyzed to assess changes in feeding strategies of the corals. Both species acquired nutrients by heterotrophic feeding and translocation from symbionts. Colonies of *M. cavernosa* acquired photosynthetic nutrients in shallow and mesophotic habitats, and the relative sterol (and phytosterol) composition did not change with depth. CSIA evidence suggests that photosynthesis slowed with increasing depth. Colonies of *Agaricia* spp. used heterotrophic feeding throughout their depth range and acquired some photosynthetic nutrients in shallow habitats and few in mesophotic habitats. Both corals, *Agaricia* spp. and *M. cavernosa*, may be able to take advantage of deep reef refugia to maintain populations in a changing ocean by using distinct nutrient acquisition strategies.

1. Introduction

1.1. Mesophotic corals

Mesophotic coral reefs are rarely studied habitats that host a variety of marine life in the depth range between 30 and 150 m (Bongaerts et al., 2010; Lesser et al., 2009, 2010; Locker et al., 2010; Sherman et al., 2010; Slattery et al., 2011). Photosynthetically active radiation (PAR) levels at these depths are considerably less (1–10% of surface irradiance) than shallow habitats, yet photosynthetic organisms populate these mesophotic reefs and photosynthesis can be productive in these lower light habitats (Falkowski et al., 1990) due in part to increased chlorophyll content (Kaiser et al., 1993; Lesser, 2000; Titlyanov et al., 2001). Vertical connectivity (Kahng et al., 2014; Serrano et al., 2014) and deep reef refugia (Riegl and Piller, 2003) may be important to the future health and resilience of coral reefs. These deeper environments, where photosynthesis can still play a significant role, are suggested to be refuges for generalist reef corals that grow in deeper waters that could repopulate shallow reefs where climate change induced extreme weather events cause mass mortality events in shallow ecosystems (Bongaerts et al., 2010; Riegl and Piller, 2003; Slattery et al., 2011).

Mesophotic reefs have significant populations of symbiotic hermatypic scleractinian corals including *Montastraea cavernosa* and *Agaricia* spp. (Bongaerts et al., 2013; Kahng et al., 2010; Lesser et al., 2009; Muscatine et al., 1989; Rooney et al., 2010). At these lower light levels, some scleractinian corals are mixotrophic and can acquire their nutrients from either translocation from their symbionts or from direct feeding on picoplankton, zooplankton, and other heterotrophic food sources. In mesophotic Caribbean reefs, internal waves or tidal bores can deliver nutrients to benthic organisms (Leichter et al., 2003) and corals (Leichter and Genovese, 2006). Although these two corals have distinct morphologies, they are abundant across a wide depth range indicating that they are well equipped for survival in varying light conditions. The foliaceous *Agaricia* spp. may be particularly adapted to maximize its surface area for prey capture, while the mounding and plating *M. cavernosa* may optimize its morphology for light collection (Lesser et al., 2010).

Other mechanisms that *M. cavernosa* employs to maximize photosynthesis in mesophotic habitats include increased photosynthetic pigments (Lesser, 2000) and light scattering techniques to maximize photon efficiency (Enriquez et al., 2005). Colonies of *M. cavernosa* can change their type of symbionts across a depth range (Lesser et al., 2010), presumably to maximize the acquisition of photosynthetic nutrients and energy.

Corals that grow and thrive in mesophotic environments may be uniquely adapted for the different light conditions. Lipid content appears to be a species-specific trait with lipid levels in different scleractinian corals varying widely (Harland et al., 1993). In a study where light levels were controlled, the lipid levels of *Pocillipora damicornis* were sensitive to light and decreased within a matter of days when shielded from light (simulating mesophotic conditions)

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http://dx.doi.org/10.1016/j.jembe.2015.10.010
0022-0981/

and recovered lipid levels soon after light shields were removed (Stimson, 1987). *Porites porites* growing near mesophotic depths had significantly lower lipid levels than shallow colonies in the Caribbean (Harland et al., 1992).

1.2. Stable carbon and nitrogen isotopes

The stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope compositions of organisms are commonly used to study feeding behaviors and have been used to study the nutrient acquisition strategies of scleractinian corals over a range of depths. Bulk analysis of the coral holobiont, which includes the coral animal and associated symbionts, and compound specific stable isotope analysis (CSIA) of individual biomarker lipid compounds including fatty acids and sterols (Teece et al., 2011; Tolosa et al., 2011; Treignier et al., 2009) are techniques employed to determine nutrient acquisition by scleractinian corals.

Numerous studies show that the $\delta^{13}C$ values of both coral tissue and symbionts consistently decrease with increasing depth (Alamaru et al., 2009; Einbinder et al., 2009; Lesser et al., 2010; Maier et al., 2010; Mass et al., 2007; Muscatine and Kaplan, 1994; Muscatine et al., 1989), however not all researchers agree that decreasing $\delta^{13}C$ in tissue represents evidence for a shift to heterotrophy. In some species, the difference between the $\delta^{13}C$ values of the two symbiotic partners increased with depth suggesting that corals were becoming less dependent on their symbionts as a source of carbon with increasing depth (Lesser et al., 2010; Muscatine et al., 1989). In contrast, Alamaru et al. (2009) found that the decrease in the $\delta^{13}C$ values of *Stylophora pistillata* larvae may be due to lower photosynthetic rates at depth rather than a greater reliance on heterotrophy because they do not capture prey.

The $\delta^{15}N$ values of scleractinian corals can be used to determine the dominant feeding strategy and trophic level at which corals are feeding. The $\delta^{15}N$ values of organisms correlate to their nitrogen source and become progressively more enriched (or more positive) with increasing trophic level (Peterson and Fry, 1987). The $\delta^{15}N$ values of corals can vary considerably with increasing depth. The $\delta^{15}N$ values of some coral species decrease markedly with increasing depth (Baker et al., 2011; Heikoop et al., 1998; Maier et al., 2010; Muscatine and Kaplan, 1994), whereas $\delta^{15}N$ values of other coral species vary little with depth (Alamaru et al., 2009; Lesser et al., 2010). The $\delta^{15}N$ values of gorgonians may also decrease with increasing depth, a pattern believed to be related to decreased light availability as shown by laboratory experiments that found nitrogen isotope fractionation to be negatively correlated with light intensity in gorgonians (Baker et al., 2011).

1.3. Sterols as biomarkers

Sterols are useful biomarkers for scleractinian coral studies (Treignier et al., 2008, 2009; Yamashiro et al., 1999) because they can often be traced back to their source (Volkman, 1986; Withers et al., 1982) and offer insights into how the coral acquired them. Sterols are essential biomolecules that contribute to membrane function and fluidity (Piironen et al., 2000). Most animals, including invertebrates like corals, are unable to synthesize sterols (Goad, 1981) so these molecules must be acquired through feeding or by translocation from a symbiont. Diverse sterols (phytosterols) are produced in abundance by photosynthetic organisms including algae and higher plants. This study analyzed the sterol composition and stable carbon isotope values of individual sterols to provide information about their source and to identify nutrient acquisition strategies of two coral species in shallow and mesophotic habitats. In particular, the $C_{28}\Delta^5$ sterol (campesterol) (Treignier et al., 2009) and the C_{30} phytosterols are biomarker molecules that are only produced by the dinoflagellate symbionts (Withers et al., 1982). Zooplankton, an important food source for corals, typically contain high amounts of $C_{27}\Delta^5$ sterol (cholesterol) (Treignier et al., 2009), so $C_{27}\Delta^5$ is a biomarker for heterotrophy. The C_{29} sterols are generally viewed as phytosterols produced through photosynthesis, but they are also found at low levels in marine zooplankton (Treignier et al., 2009) and phytoplankton (Lin et al., 1982) so they can be acquired through both feeding and translocation.

Stable isotope analysis of the separated host and symbiont record the $\delta^{13}C$ values of the overall carbon metabolism of these organisms but are limited in assessing the source of translocated or heterotrophically acquired carbon materials. Measuring the $\delta^{13}C$ values of specific lipid compounds (CSIA), that have known sources or biochemical functions, provides an elegant way to assess nutrient acquisition in corals (Teece et al., 2011; Tolosa et al., 2011; Treignier et al., 2009) that has several advantages. CSIA of biomarker sterols that are specific to symbionts provides a means to determine their source in corals. Sterol abundance profiles were proposed as a useful way to distinguish coral species (Yamashiro et al., 1999) and they have served as biomarker compounds to determine the origin of organic matter in marine (Burns et al., 2003; Rontani et al., 2011; Volkman and Tanoue, 2002) and estuarine (McCallister et al., 2006; Zimmerman and Canuel, 2002) environments. Similar CSIA approaches were successfully used to assess dietary sources of giant clams (Johnston et al., 1995), sea urchins, (Villinski et al., 2004) and hydrothermal vent organisms (Rieley et al., 1999). Compound specific isotope analysis of known biomarker compounds, distinct to translocation from symbiont to host, can indicate the utilization of these compounds in corals (Treignier et al., 2009).

Lipids, including sterols, are typically depleted in ^{13}C relative to the bulk tissue. These lipids are expected to be depleted relative to the bulk tissue because of carbon fractionation in the biosynthetic pathway due to pyruvate dehydrogenase fractionation (DeNiro and Epstein, 1977). This depletion is observed in coral fatty acids (Teece et al., 2011) and in coral sterols (Treignier et al., 2009).

Comparison of sterol profiles, bulk stable isotopes, and CSIA of individual sterols can provide important insights into the nutrient acquisition behavior of corals in the mesophotic zone. Sterols are particularly well suited as biomarkers for nutrient acquisition because their origin can be identified. The objectives of this study were to use these techniques to assess the nutrient acquisition strategies of two scleractinian corals, The colonies of *M. cavernosa* and *Agaricia* spp. were collected from shallow (depths less than 20 m) and mesophotic (depths of 30–150 m) habitats.

2. Materials and methods

2.1. Sample collection

Trained technical divers collected coral chips at “Rock Bottom” (19° 41.67′ N, 80° 4.17′ W) and “Paul's Anchors” (19° 42.00′ N, 80° 3.42′ W) located on the north wall near the Little Cayman Research Centre using SCUBA. Samples of *M. cavernosa* and *Agaricia* spp. were collected from a shallow zone at 18–20 m and from the mesophotic zone at 55–60 m at both sites. Samples were packed in labeled vials and returned promptly to the laboratory where the top 4 mm of coral holobiont (animal and symbiont) tissue was removed from the coral chip by scraping it off. An aliquot of the holobiont tissue was filtered (described below) for bulk stable isotope analysis. The remaining holobiont tissue was then frozen and lyophilized (−60 °C, 48 h, 150 Torr) for subsequent organic matter extraction. Eight colonies of shallow and nine colonies of mesophotic *M. cavernosa* were analyzed for their bulk stable isotope values. Four colonies of shallow and five colonies of mesophotic *Agaricia* spp. were analyzed for their bulk stable isotope values. In deeper waters, species identification of *Agaricia* spp. becomes difficult because the different species (*A. lamarki* and *A. agaricites*) are morphologically similar (Bongaerts et al., 2013; Wells, 1973). Compound specific stable isotope analysis and composition analysis of sterols was performed on a subset of these colonies (2–4 each).