Journal of the Marine Biological Association of the United Kingdom, 2016, 96(2), 559–565.
doi:10.1017/S0025315415000363

Niche structure of marine sponges from temperate hard-bottom habitats within Gray's Reef National Marine Sanctuary

CHRISTOPHER J. FREEMAN[1], COLE G. EASSON[2] AND DAVID M. BAKER[3]

[1]Smithsonian Marine Station, Fort Pierce, FL, USA, [2]Department of Biology, University of Alabama at Birmingham, Birmingham, AL, USA, [3]The Swire Institute of Marine Science, School of Biological Sciences & Department of Earth Science, University of Hong Kong, Hong Kong, PR China

Many species of marine sponges on tropical reefs host abundant and diverse symbiont communities capable of varied metabolic pathways. While such communities may confer a nutritional benefit to some hosts (termed High Microbial Abundance (HMA) sponges), other sympatric species host only sparse symbiont communities (termed Low Microbial Abundance (LMA) sponges) and obtain a majority of their C and N from local sources. Sponge communities are widespread across large latitudinal gradients, however, and recent evidence suggests that these symbioses may also extend beyond the tropics. We investigated the role that symbionts play in the ecology of sponges from the temperate, hard-bottom reefs of Gray's Reef National Marine Sanctuary by calculating the niche size (as standard ellipse area (SEA_c)) and assessing the relative placement of five HMA and four LMA sponge species within bivariate ($\delta^{13}C$ and $\delta^{15}N$) isotopic space. Although photosymbiont abundance was low across most of these species, sponges were widespread across isotopic niche space, implying that microbial metabolism confers an ecological benefit to temperate sponges by expanding host metabolic capability. To examine how these associations vary across a latitudinal gradient, we also compared the relative placement of temperate and tropical conspecifics within isotopic space. Surprisingly, shifts in sponge $\delta^{13}C$ and $\delta^{15}N$ values between these regions suggest a reduced reliance on symbiont-derived nutrients in temperate sponges compared with their tropical conspecifics. Despite this, symbiotic sponges in temperate systems likely have a competitive advantage, allowing them to grow and compete for space within these habitats.

Keywords: Gray's Reef, latitudinal gradient, isotopic niche space, microbes, microbial abundance, Porifera, standard ellipse area, stable isotopes, symbiosis, temperate

Submitted 31 October 2014; accepted 10 March 2015; first published online 10 April 2015

INTRODUCTION

Microbial symbionts increase the metabolic diversity of many eukaryotes, allowing their hosts to survive and compete for space in systems that would otherwise be inhospitable (Moran, 2007; Moya *et al.*, 2008). Indeed, the products of autotrophic and chemosynthetic symbiont metabolism sustain diverse ecosystems on tropical coral reefs and around deep-sea hydrothermal vents, respectively (Muscatine & Cernichiari, 1969; Vrijenhoek, 2010). On oligotrophic coral reefs, these interactions are exemplified by the mutualisms between photosynthetic symbionts and reef-building corals, octocorals and sponges (Muscatine & Porter, 1977; Wilkinson, 1983; Freeman & Thacker, 2011; Baker *et al.*, in review). These symbioses are incredibly complex, with substantial diversity both across functional groups and across host species (Knowlton & Rohwer, 2003; Thacker & Freeman, 2012; Freeman *et al.*, 2013; Baker *et al.*, in review).

In marine sponges, some species support symbiont communities spanning almost all evolutionary lineages of bacteria and archaea (Thacker & Freeman, 2012). These high microbial abundance (HMA) species have long been hypothesized to derive substantial nutritional benefit from these associations (Taylor *et al.*, 2007). Other sympatric sponge species hosting only sparse symbiont communities (termed LMA (Low Microbial Abundance)) may rely more heavily on heterotrophic filter feeding to meet their energy demands (Weisz *et al.*, 2007; Freeman & Thacker, 2011). Recent research, however, has shown that variation in symbiont-derived benefit may be driven more by the presence of specific, physiologically unique symbiont groups than overall symbiont abundance (Freeman *et al.*, 2013). Thus, while hosting symbionts may allow HMA species to utilize sources of C and N that are unavailable to most LMA species, the evolution of highly specific host-symbiont associations (termed holobionts; Easson & Thacker, 2014) may lead to substantial variation in biogeochemical C and N cycling across sympatric sponge species (Taylor *et al.*, 2007; Mohamed *et al.*, 2008; Southwell *et al.*, 2008; Maldonado *et al.*, 2012). Indeed, broad dispersion of individual species within bivariate ($\delta^{13}C$ and $\delta^{15}N$) isotopic 'niche space' suggests that there is a complex niche structure in tropical sponge communities (Newsome *et al.*, 2007; Freeman *et al.*, 2014).

Sponge communities extend into temperate and polar latitudes, however, and recent evidence supports the contention

Corresponding author:
C.J. Freeman
Email: freemanc@si.edu

that these symbioses also extend beyond the tropics (Webster *et al.*, 2004; Becerro, 2008; Erwin *et al.*, 2012). For instance, in temperate Western Australia, 48% of the sponge species surveyed using PAM fluorometry were categorized as positive for the presence of photosymbionts (Lemloh *et al.*, 2009). Photosymbionts have also been reported in sponges from additional temperate sites in Australia, Ireland and the Mediterranean (Roberts *et al.*, 1999; Bell, 2007; Erwin *et al.*, 2012). While these symbiont communities may allow some sponges to utilize inorganic sources of C and N (Weisz, 2006), little is known about the role these associations play in host ecology within temperate systems and even less is known about how these interactions may change over latitudinal gradients (Muller-Parker & Davy, 2001; Usher, 2008).

To investigate the role these associations play in host sponge ecology in temperate systems, we adapted the isotopic niche space concept, which posits that the relative placement of an organism in two-dimensional ($\delta^{13}C$ and $\delta^{15}N$) isotopic space is a reliable indicator of the physiochemical 'niche' space it fills in a system (Newsome *et al.*, 2007; Thurber, 2007; Layman *et al.*, 2012). Thus, because the niche position of a consumer is driven by both the sources of C and N assimilated and biochemical processing of these sources (Newsome *et al.*, 2007; Layman *et al.*, 2012), we would expect wide disparity in the placement of sympatric sponge species across isotopic niche space if microbial symbionts expand the metabolic capabilities of their host. These methods allowed us to quantitatively depict and compare the niche space filled by nine sponge species from the temperate hard-bottom habitats within Gray's Reef National Marine Sanctuary (GRNMS) (Jackson *et al.*, 2011; Layman *et al.*, 2012). The placement of each species in isotopic niche space was compared with its photosymbiont abundance (as measured by chlorophyll *a*) and its characterization as either an HMA or LMA species. In addition, because sponges within GRNMS have tropical Caribbean conspecifics (Freeman *et al.*, 2007), we also investigated how these associations change with latitude by comparing the isotopic niche space occupied by sponges from GRNMS to data from the same species collected previously from tropical reefs in Honduras (Freeman *et al.*, 2014).

MATERIALS AND METHODS

Study site and species collected

Sponges were sampled across 10 sites within Gray's Reef National Marine Sanctuary (GRNMS; 31°23.815N, 80°53.461W) during a June 2013 research cruise aboard the NOAA RV Nancy Foster. Gray's Reef is a temperate, hard-bottom reef approximately 25 km off the coast of Georgia, USA. These limestone, sandstone or relic scallop shell reefs include ledges and escarpments that provide substrate for diverse assemblages of tropical and temperate benthic organisms, including over 50 species of sponges (Freeman *et al.*, 2007; Ruzicka & Gleason, 2009). Unlike tropical coral reefs, however, the benthic community within GRNMS is strikingly heterotrophic, with a heavy reliance on inputs of allochthonous organic carbon to meet respiratory requirements (Hopkinson *et al.*, 1991).

We collected individuals of the sponge species *Aiolochroia crassa* (Hyatt, 1875); *Aplysina fulva* (Pallas, 1766); *Chondrilla caribensis*, Rützler, Duran & Piantoni, 2007; *Cinachyrella alloclada* (Uliczka, 1929); *Desmapsamma anchorata* (Carter, 1882); *Dysidea fragilis* (Montagu, 1814); *Ircinia campana* (Lamarck, 1814); *Ircinia felix* (Duchassaing & Michelotti, 1864); and *Scopalina ruetzleri* (Wiedenmayer, 1977). These species were chosen because they are abundant within GRNMS, have tropical conspecifics in the Caribbean, and include members of both HMA (*A. crassa, A. fulva, C. caribensis, I. campana* and *I. felix*) and LMA (*C. alloclada, D. anchorata, D. fragilis* and *S. ruetzleri*) groups (Thacker & Freeman, 2012; Freeman *et al.*, 2014; Gloeckner *et al.*, 2014; Supplementary Table 1). At each site, individuals of these species were collected when present, along with a sample of seawater from depth for an assessment of the $\delta^{13}C$ and $\delta^{15}N$ values of particulate organic matter (POM), a potential source of C and N for sponges feeding heterotrophically (Freeman & Thacker, 2011). Individuals of the following species were collected at multiple sites: *A. crassa, A. fulva, C. caribensis, I. campana, I. felix* and *S. ruetzleri*, while *C. alloclada, D. anchorata* and *D. fragilis* were relatively less abundant within GRNMS and were thus only collected from a single site. All sites were within a 28 km^2 area of GRNMS and had an depth between 18–20 m. After each dive, sponge specimens were catalogued, potential contaminants (such as macroalgae embedded within *Scopalina ruetzleri*) were removed with forceps, and tissue was immediately frozen at −20°C; water samples were filtered through a 0.70 μm GF filter to obtain POM as in Freeman & Thacker (2011) and also frozen at −20°C for future analyses.

Isotope and chlorophyll *a* analysis

At the Smithsonian Marine Station in Fort Pierce, FL, USA, sponge samples were lyophilized and ground to a fine powder using a mortar and pestle. Homogenized sponge tissue and GF filters were acidified to remove carbonate and weighed into tared silver capsules for $\delta^{13}C$ and $\delta^{15}N$ analysis as in Freeman & Thacker (2011). Sponge and POM samples were analysed in the Stable Isotope Ratio Mass Spectrometry laboratory (SIRMS) at the University of Hong Kong via combustion in a Eurovector EA3028 coupled to a Perspective IRMS (Nu Instruments). Analytical precision was determined by repeated analysis of an internal acetanilide standard ('acet 6'; 70% C). Mean (±SE) precision during analysis was 0.2 ± 0.04 and 0.1 ± 0.01 for $\delta^{15}N$ and $\delta^{13}C$, respectively. Isotope data are expressed in delta (δ) or permil (‰) notation as in Freeman & Thacker (2011) and as described in Fry (2006). Photosymbiont abundance (chlorophyll *a* (chl *a*)) analyses were carried out on lyophilized tissue as in Freeman & Thacker (2011) and Freeman *et al.* (2013).

Data analysis

The niche structure of these nine sponge species from GRNMS was estimated by calculating the standard ellipse area (SEA_c) of each species using a Bayesian approach based on multivariate ellipse-based metrics (SIBER (Stable Isotope Bayesian Ellipses in R); Jackson *et al.*, 2011). Because the SEA_c contains approximately 40% of the data within a set of bivariate ($\delta^{13}C$ and $\delta^{15}N$) data, an ellipse represents the core niche area for a population or community (Jackson *et al.*, 2011; Layman *et al.*, 2012). Unlike other estimates of niche width (the area of a convex hull enclosing all data points (Total Area, TA); Layman *et al.*, 2007), SEA_c calculations are less susceptible to outliers. In addition, using Bayesian inference allows for more robust comparisons across sets of

https://doi.org/10.1017/S0025315415000363 Published online by Cambridge University Press

data with variable sample sizes. We adapted these methods to visualize the placement of each of these species within the isotopic 'niche space' of GRNMS. We also compared the placement of individual species within this isotopic space using methods outlined by Turner *et al.* (2010). These methods calculate the Euclidean distance between the centroids (bivariate mean) of individual species within isotopic space and use a residual permutation procedure (RPP) and Hotelling T^2 test to evaluate whether this distance is significant (different from zero), thus placing these individual species in unique isotopic space (Turner *et al.*, 2010).

In addition, to assess how sponge niche structure changes across a tropical-temperate gradient, we calculated the niche area (as SEA_c) and assessed the relative position of temperate and tropical sponge communities. These communities included individuals of *A. crassa, A. fulva, C. caribensis, D. anchorata* and *I. campana* collected from the temperate reefs of GRNMS and tropical reefs within the Miskito Cays of Honduras (Chollett *et al.*, 2014). Data for sponge communities from Honduras were collected previously as part of a prior study (Freeman *et al.*, 2014). We generated a SEA_c from each of these communities using the mean values for each of these five species (Jackson *et al.*, 2011; Layman *et al.*, 2012). Above statistical analyses were conducted in R v. 3.1.1 using R commands adapted from Turner *et al.* (2010) and Jackson *et al.* (2011). We also assessed the relative effects of host species identity and site (GRNMS *vs* Honduras) on the placement of samples within isotopic space using the function adonis in the R package vegan (Oksanen *et al.*, 2014; as in Freeman *et al.*, 2014).

We conducted an Analysis of Variance (ANOVA) to test for differences in the $\delta^{13}C$ and $\delta^{15}N$ values and chl *a* concentrations of sponge species collected from disparate sites within GRNMS. As these values were similar ($P > 0.05$) across sites, isotope values and chl *a* concentrations were pooled for each species prior to further analyses. We also used an ANOVA to test for differences in the $\delta^{13}C$ and $\delta^{15}N$ values of POM, sponge communities, and individual sponge species from GRNMS and Honduras. Prior to SIBER analyses on isotope data, residuals were tested for normality and homogeneity of variances among groups. These statistical analyses were conducted using Systat v. 11 (Systat, Inc.).

RESULTS

Photosymbiont abundance (measured by chl *a*) ranged from approximately 280 μg chl *a* (g AFDW of sponge tissue^{-1}) in *Scopalina ruetzleri* to 15 μg chl *a* (g AFDW of sponge tissue^{-1}) in *Aiolochroia crassa* (Figure 1). Although these nine species include members of both HMA and LMA groups, and many of these HMA species typically have chl *a* concentrations well above 200 μg chl *a* (g AFDW of sponge tissue^{-1}) in the Caribbean (Freeman *et al.*, 2013, 2014; Supplementary Table 1), almost all species (except the LMA species *S. ruetzleri*) had chl *a* values that were below 150 μg chl *a* (g AFDW of sponge tissue^{-1}).

SIBER analysis showed low overlap of the standard ellipse areas (SEA_c) of the nine species at GRNMS (Figure 2) and host identity accounted for over 90% of the variance in sample placement within isotopic space (adonis: $F = 133.7$, $R^2 = 0.93$, $P < 0.001$). In fact, while the SEA_c of *Dysidea fragilis* and *S. ruetzleri* overlapped by approximately 7%, and these species occupied a similar location in isotopic niche space (distance between centroids = 0.26; Hotelling's T test: $T^2 = 2.30$, $F = 0.88$, $P = 0.38$; Turner *et al.*, 2010), the SEA_c of the other seven species did not overlap and each species was present within unique isotopic niche space ($P < 0.05$). With $\delta^{15}N$ values ranging from ~7.0 to 9.4‰, the LMA species were generally more enriched in ^{15}N than the HMA species ($\delta^{15}N$ values from 2.2 to 8.2‰, with only *Chondrilla caribensis* above 7.0‰; Figure 2). In addition, the $\delta^{13}C$ values of LMA

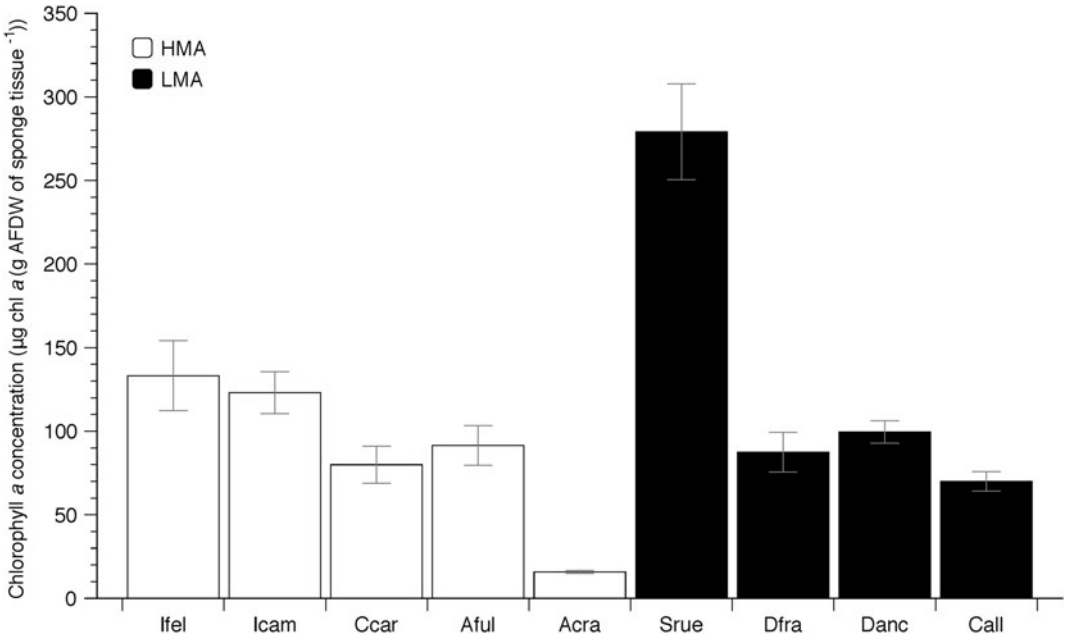

Fig. 1. Mean chlorophyll *a* (chl *a*) concentration (±SE) of nine sponge species from Gray's Reef National Marine Sanctuary. Sponges include members of Low (LMA – filled columns) and High (HMA – open columns) Microbial Abundance groups (Weisz, 2006; Freeman *et al.*, 2014; Gloeckner *et al.*, 2014). Abbreviations represent the first letter of the genus name, followed by the first three letters of the specific epithet.

https://doi.org/10.1017/S0025315415000363 Published online by Cambridge University Press

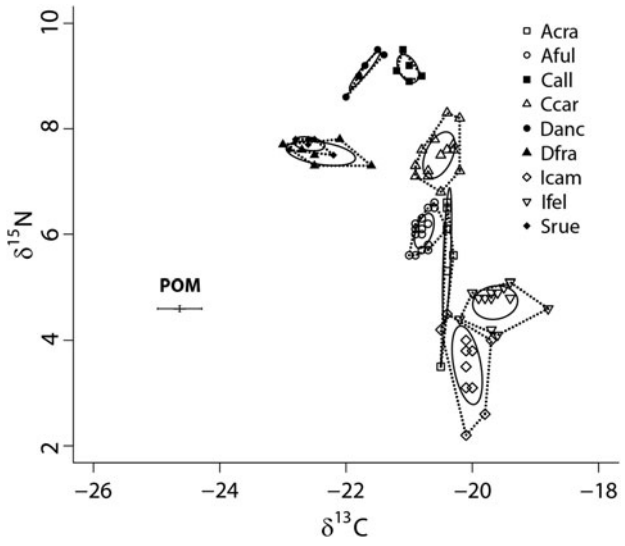

Fig. 2. Bivariate $\delta^{13}C$ and $\delta^{15}N$ plot depicting the placement of nine sponge species within the isotopic niche space of Gray's Reef National Marine Sanctuary. Sponges include members of Low (filled symbols) and High (open symbols) Microbial Abundance groups (Weisz, 2006; Freeman *et al.*, 2014; Gloeckner *et al.*, 2014). Standard ellipse areas (SEA_c) depicted by solid lines provide estimates of the niche area of each of these species using Bayesian inference as in Jackson *et al.* (2011). Convex hulls depicting total niche width of each species are also shown using dashed lines for reference (Layman *et al.*, 2007). Species abbreviations are the same as in Figure 1. Mean particulate organic matter (POM) $\delta^{13}C$ and $\delta^{15}N$ values (±SE) for GRNMS are also shown.

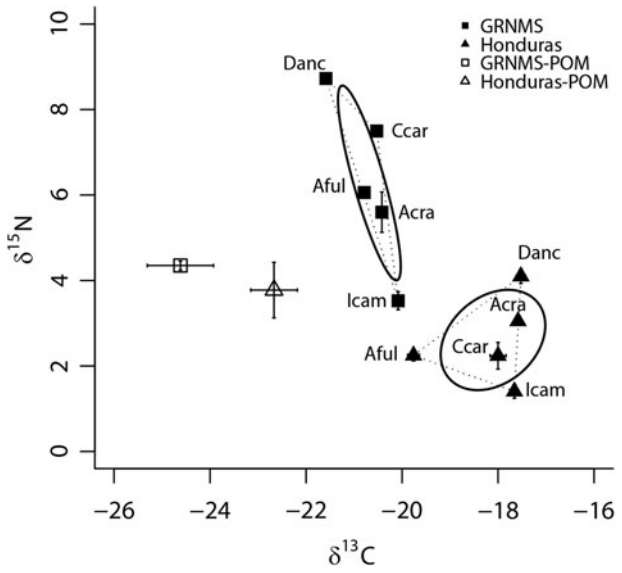

Fig. 3. Bivariate $\delta^{13}C$ and $\delta^{15}N$ plot depicting the placement of five sponge species from temperate Gray's Reef National Marine Sanctuary (GRNMS; filled squares) and tropical Honduras (filled triangles; from Freeman *et al.*, 2014) within isotopic niche space. Mean values (±SE) are shown for each species. Standard ellipse areas (SEA_c) depicted by solid lines provide estimates of the niche area of each of these communities using Bayesian inference as in Jackson *et al.* (2011). Convex hulls depicting total niche width of each community are also shown using dashed lines for reference (Layman *et al.*, 2007). Mean (±SE) $\delta^{13}C$ and $\delta^{15}N$ for particulate organic matter (POM) from GRNMS (open square) and Honduras (open triangle) are also shown. Species abbreviations are the same as in Figures 1 and 2.

species were generally depleted in ^{13}C relative to HMA species (−20.8 to −23.0‰ *vs* −18.8 to −21‰ for LMA and HMA, respectively; Figure 2). Although there was some variation in SEA_c size across these nine species, we purposefully limit our discussion of these data, as some, but not all, of these sponges were collected at multiple sites. We were unable to obtain accurate $\delta^{13}C$ and $\delta^{15}N$ values of POM from all 10 sites within GRNMS due to lower organic matter on some filters. There was, however, minimal variation in the $\delta^{13}C$ and $\delta^{15}N$ values of particulate organic matter (POM) across six sites, with mean (±SE) values around −24.6 ± 0.7 and 4.3 ± 0.1‰, respectively.

Although the SEA_c of the sponge communities from GRNMS and Honduras were similar in size ($P = 0.39$), each of these communities was present within unique isotopic niche space (0% overlap; distance between centroids = 4.48; Hotelling's T test: $T^2 = 32.86$, $F = 11.50$, $P < 0.01$; Figure 3), and these communities had significantly different $\delta^{13}C$ and $\delta^{15}N$ values (mean (±SE): −20.6 ± 0.1 and −18.6 ± 0.2‰ for $\delta^{13}C$ of GRNMS and Honduras, respectively; 6.2 ± 0.2 and 2.2 ± 0.1‰ for $\delta^{15}N$ of GRNMS and Honduras, respectively; ANOVA: $F = 150.7$, $P < 0.0001$, and $F = 208.4$, $P < 0.0001$ for $\delta^{13}C$ and $\delta^{15}N$, respectively). In addition, when considering data from both sites, host species identity accounted for 37% (adonis: $F = 15.2$, $R^2 = 0.37$, $P < 0.001$) of the variance in sample placement within isotopic space, whereas site accounted for over 60% (adonis: $F = 192.8$, $R^2 = 0.65$, $P < 0.001$) of this variance.

The $\delta^{13}C$ values of both POM (ANOVA: $F = 5.4$, $P = 0.04$) and all five sponge species (ANOVA: $P < 0.05$) were significantly depleted at GRNMS compared with Honduras (Figure 3; Table S1). In contrast, although the $\delta^{15}N$ values of all five sponge species were significantly more enriched at GRNMS (ANOVA: $P < 0.05$) than in Honduras, there was no significant difference in the $\delta^{15}N$ values of POM between these two sites (ANOVA: $F = 0.7$, $P = 0.41$; Figure 3; Table S1). The sponge community from GRNMS was, on average, 1.9‰ enriched in $\delta^{15}N$ relative to POM and almost all species (except *Ircinia campana* (0.8‰ depleted in $\delta^{15}N$ relative to POM)) were enriched in $\delta^{15}N$ (between 0.4 and 4.8‰) relative to POM. The sponge community from Honduras, on the other hand, was, on average, 1.2‰ depleted in $\delta^{15}N$ relative to POM and almost all species (except *Desmapsamma anchorata* (0.4‰ enriched in $\delta^{15}N$ relative to POM)) were depleted in $\delta^{15}N$ (between 0.6 and 2.3‰) relative to POM (Figure 3).

DISCUSSION

Unlike in the tropics, photosymbiont abundance values (chlorophyll *a*) in sponges from GRNMS were generally low (<150 μg chl *a* (g AFDW of sponge tissue^{-1})), regardless of whether these species were categorized as HMA or LMA. In fact, the average (±SE) chl *a* concentration of the five HMA species in this study was approximately 90 (±20) μg chl *a* (g AFDW of sponge tissue^{-1}), whereas the mean chl *a* concentration for these same HMA species was around 320 (±100) μg chl *a* (g AFDW of sponge tissue^{-1}) in tropical Honduras (Freeman *et al.*, 2014), and 160 (±40) μg chl *a* (g of sponge tissue^{-1}) in Panama (Erwin & Thacker, 2007). Although the LMA species *Scopalina ruetzleri* had abnormally elevated chl *a* concentrations (>250 μg chl *a* (g AFDW of

https://doi.org/10.1017/S0025315415000363 Published online by Cambridge University Press

sponge tissue^{-1}) at GRNMS compared with only 40 μg chl *a* (g of sponge tissue^{-1}) in Panama; Erwin & Thacker, 2007), these levels likely reflect the fact that this semi-encrusting species grows in close proximity to and around some macroalgae at GRNMS (see photos at GRNMS benthic invertebrate field guide: http://www.bio.georgiasouthern.edu/gr-inverts/index.html). While we removed apparent algal tissue following collection and prior to chl *a* and isotope analyses, deeply embedded algae may have amplified these chl *a* values. Therefore, unlike the other species, chl *a* values in *S. ruetzleri* likely do not reflect true photosymbiont abundance.

Although photosymbiont populations were reduced in HMA sponges from these temperate reefs (Muller-Parker & Davy, 2001), striking disparity in the placement of sponges within the isotopic niche space of GRNMS suggests that biogeochemical cycling of C and N is highly variable across these nine species (Newsome *et al.*, 2007; Layman *et al.*, 2012). Microbial communities may allow HMA holobionts to assimilate sources of C and N that are unavailable to LMA species within this system (Weisz *et al.*, 2007; Thacker & Freeman, 2012; Freeman *et al.*, 2014). Indeed, the placement of LMA species in isotopic space relative to HMA species and particulate organic matter (POM: $\delta^{13}C$ and $\delta^{15}N$ values of -24.6 ± 0.7 and 4.3 ± 0.1‰, respectively) suggest that LMA species may be more reliant on local sources of C and N than most HMA species (Michener & Kaufman, 2007; Weisz *et al.*, 2007; Freeman & Thacker, 2011). Substantial dispersion of HMA species and the strong effect of host species identity in the placement of individual samples across isotopic space makes such broad generalizations difficult, however, and implies that factors other than overall symbiont abundance are driving the relative placement of these species in isotopic niche space (Freeman *et al.*, 2014).

The placement of individual LMA species may reflect specialization on particular size fractions of the POM pool or the assimilation of abundant dissolved organic matter (DOM; Hopkinson *et al.*, 1991) by sponge cells or microbes within some species (Thurber, 2007; Maldonado *et al.*, 2012; Easson & Thacker, 2014). The relative placement of HMA species, on the other hand, may reflect variation in symbiont metabolism, relative host reliance on symbiont- *vs* heterotrophically derived nutrients, or internal transformations and cycling of nutrients within holobionts of different species (Newsome *et al.*, 2007; Taylor *et al.*, 2007; Southwell *et al.*, 2008; Thacker & Freeman, 2012). For example, photosymbionts may allow some holobionts to fix inorganic sources of C, but photosymbiont productivity may vary substantially across diverse holobionts (Erwin & Thacker, 2007, 2008; Freeman *et al.*, 2013). In addition, some species may host abundant microbial communities, but maintain a reliance on C and N from both microbial metabolism and heterotrophic feeding on local sources (Freeman & Thacker, 2011). Microbial communities within other species may further expand the metabolic repertoires of their host by fixing and further transforming inorganic sources of N (Mohamed *et al.*, 2008; Maldonado *et al.*, 2012); largely heterotrophic microbial symbionts in additional species may assimilate diverse and abundant sources of dissolved organic matter (Hopkinson *et al.*, 1991; van Duyl *et al.*, 2011). While our data provide limited information on the relative contribution of different sources to each sponge host, the expansion of these species across isotopic niche space implies that microbial symbionts increase host metabolic diversity and thus likely play an important role in host ecology, even on temperate reefs (Knowlton & Jackson, 1994).

The trophic position of this sponge community shifted considerably across a tropical-temperate gradient. At GRNMS, almost all (except *Ircinia campana*) of these species were enriched in $\delta^{15}N$ relative to POM, while on tropical reefs most (except *Desmapsamma anchorata*) of these sponges were depleted relative to this potential heterotrophic food source. Because the process of trophic enrichment leads to consumers that are generally enriched in $\delta^{15}N$ compared with their prey (Michener & Kaufman, 2007), we propose that there is a general trend towards a reduction in sponge reliance on symbiont-derived nutrients in temperate conspecifics, with a concurrent increase in the assimilation of local, diverse nutrient sources (Hopkinson *et al.*, 1991; Michener & Kaufman, 2007; Weisz *et al.*, 2007; Freeman & Thacker, 2011).

Shifts in POM $\delta^{13}C$ values across this latitudinal gradient may be driven by variation in the ultimate C source supporting food webs in each of these regions. For instance, while benthic communities near (26 km offshore) coastal GA may be largely supported by terrestrial sources of C with depleted $\delta^{13}C$ values, communities far (~70 km) off the coast of Honduras are probably supported by marine sources of C with more enriched $\delta^{13}C$ values (Hopkinson *et al.*, 1991; Deegan & Garritt, 1997; Michener & Kaufman, 2007; Lamb & Swart, 2008). Although sponge $\delta^{13}C$ values from both communities are well outside the range expected by sponges feeding exclusively on bulk POM (POM $\delta^{13}C$ value + 0.5 to 1.0‰; Michener & Kaufman, 2007), depleted $\delta^{13}C$ values in both POM and sponges from GRNMS suggests a reliance on these allochthonous sources of C, and may imply a reduced dependence on photosynthetic assimilation of dissolved inorganic carbon (DIC) from plentiful seawater bicarbonate, which has a $\delta^{13}C$ value of ~0‰ (Fry, 2006).

These data provide initial evidence that symbiont communities within temperate sponges may contribute less to holobiont metabolism than tropical conspecifics (Muller-Parker & Davy, 2001; Usher, 2008). While this may be driven in part by comparatively low photosymbiont abundance in sponges at GRNMS, additional studies are needed that experimentally assess differences in photosymbiont productivity across a latitudinal gradient and investigate the relative contribution of diverse sources of C and N to hosts in both regions (Hopkinson *et al.*, 1991; Thacker & Freeman, 2012). In addition, there may be substantial shifts in photosymbiont (Erwin & Thacker, 2007; Usher, 2008) and overall microbial (Easson & Thacker, 2014) diversity and community composition across these gradients. Future studies investigating the role that these shifts play in the biogeochemical cycling of C and N within sponge holobionts are warranted.

In conclusion, although sponges at GRNMS may be less reliant on symbiont metabolism than their tropical conspecifics, the disparate placement of HMA species across isotopic niche space (Freeman *et al.*, 2014) implies that symbionts increase host metabolic diversity in these systems and allow their hosts to expand into novel physiochemical niches (Easson & Thacker, 2014; Freeman *et al.*, 2014). Although potentially minor compared with tropical conspecifics, such a nutritional benefit may provide a competitive advantage to symbiotic sponges at GRNMS, possibly enhancing growth (Muller-Parker & Davy, 2001; Usher, 2008) and allowing these species to compete for space on the densely colonized

https://doi.org/10.1017/S0025315415000363 Published online by Cambridge University Press

scarp habitat, where all five of these HMA species are found (Ruzicka & Gleason, 2009).

Supplementary materials and methods

To view supplementary material for this article, please visit http://dx.doi.org/S0025315415000363

ACKNOWLEDGEMENTS

We thank the staff of NOAA Gray's Reef National Marine Sanctuary and the crew of the NOAA RV Nancy Foster for their assistance and logistical support. In particular, we thank G. McFall and S. Fangman for their assistance with this project. M. Zhu and H. Wong assisted with isotopes and L. Concepcion, H. Hoffman, A. Nguyen and L. Hoke assisted with work in the laboratory. Sponge samples were collected under the NOAA permit GRNMS-2010-001. This is contribution #984 from the Smithsonian Marine Station at Fort Pierce.

FINANCIAL SUPPORT

Financial support for this project was provided to CJF as part of a postdoctoral fellowship with the Marine Global Earth Observatory (MarineGEO) project at the Smithsonian Institution.

REFERENCES

Becerro M.A. (2008) Quantitative trends in sponge ecology research. *Marine Ecology* 29, 167–177.

Bell J.J. (2007) The ecology of sponges in Lough Hyne Marine Nature Reserve (south-west Ireland): past, present and future perspectives. *Journal of the Marine Biological Association of the United Kingdom* 87, 1655–1668.

Chollett I., Stoyle G. and Box S. (2014) Honduran Miskito Cays: among the last unexplored reef systems in the Caribbean. *Coral Reefs* 33, 155.

Deegan L.A. and Garritt R.H. (1997) Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series* 147, 31–47.

Easson C.G. and Thacker R.W. (2014) Phylogenetic signal in the community structure of host-specific microbiomes of tropical marine sponges. *Frontiers in Microbiology* 5, 1–11.

Erwin P.M., López-Legentil S. and Turon X. (2012) Ultrastructure, molecular phylogenetics, and chlorophyll *a* content of novel cyanobacterial symbionts in temperate sponges. *Microbial Ecology* 64, 771–783.

Erwin P.M. and Thacker R.W. (2007) Incidence and identity of photosynthetic symbionts in Caribbean coral reef sponge assemblages. *Journal of the Marine Biological Association of the United Kingdom* 87, 1683–1692.

Erwin P.M. and Thacker R.W. (2008) Cryptic diversity of the symbiotic cyanobacterium *Synechococcus spongiarum* among sponge hosts. *Molecular Ecology* 17, 2937–2947.

Freeman C.J., Easson C.G. and Baker D.M. (2014) Metabolic diversity and niche structure in sponges from the Miskito Cays, Honduras. *Peer J* 2, e695. doi: 10.7717/peerj.695.

Freeman C.J., Gleason D.F., Ruzicka R., van Soest R.W.M., Harvey A.W. and Mcfall G. (2007) A biogeographic comparison of sponge fauna from Gray's Reef National Marine Sanctuary and other hard-bottom reefs of coastal Georgia, USA. In Custódio M.R., Lôbo-Hajdu G., Hajdu E. and Muricy G. (eds) *Proceedings of the Seventh International Sponge Symposium, Búzios, Brazil, May 2006. Porifera research: biodiversity, innovation, and sustainability*. Série Livros 28. Rio de Janeiro: Museu Nacional, pp. 319–325.

Freeman C.J. and Thacker R.W. (2011) Complex interactions between marine sponges and their symbiotic microbial communities. *Limnology and Oceanography* 56, 1577–1586.

Freeman C.J., Thacker R.W., Baker D.M. and Fogel M. (2013) Quality or quantity: is nutrient transfer driven more by symbiont identity and productivity than by symbiont abundance? *ISME Journal* 7, 1116–1125.

Fry B. (2006) *Stable isotope ecology*. New York: Springer.

Gloeckner V., Wehrl M., Moitinho-Silva L., Gernert C., Schupp P., Pawlik J.R., Lindquist N.L., Erpenbeck D., Wörheide G. and Hentschel U. (2014) The HMA-LMA dichotomy revisited: an electron microscopical survey of 56 sponge species. *Biological Bulletin* 227, 78–88.

Hopkinson C.S., Fallon R.D., Jansson B. and Schubauer J.P. (1991) Community metabolism and nutrient cycling at Gray's Reef, a hard bottom habitat in the Georgia Bight. *Marine Ecology Progress Series* 73, 105–120.

Jackson A.L., Inger R., Parnell A. and Bearhop S. (2011) Comparing isotopic niche widths among and within communities: SIBER-Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80, 595–602.

Knowlton N. and Jackson J.B.C. (1994) New taxonomy and niche partitioning on coral reefs: jack of all trades or master of some? *Trends in Ecology and Evolution* 9, 7–9.

Knowlton N. and Rohwer F. (2003) Multispecies microbial mutualisms on coral reefs: the host as a habitat. *American Naturalist* 162, S51–S62.

Lamb K. and Swart P.K. (2008) The carbon and nitrogen isotopic values of particulate organic material from the Florida Keys: a temporal and spatial study. *Coral Reefs* 27, 351–362.

Layman C.A., Araujo M.S., Boucek R., Harrison E., Jud Z.R., Matich P., Hammerschlag-Peyer C.M., Rosenblatt A.E., Vaudo J.J., Yeager L.A., Post D. and Bearhop S. (2012) Applying stable isotopes to examine food web structure: an overview of analytical tools. *Biological Reviews* 87, 542–562.

Layman C.A., Arrington D.A., Montaña C.G. and Post D.M. (2007) Can stable isotope ratios provide quantitative measures of trophic diversity within food webs? *Ecology* 88, 42–48.

Lemloh M., Fromont J., Brümmer F. and Usher K.M. (2009) Diversity and abundance of photosynthetic sponges in temperate Western Australia. *BMC Ecology* 9, 4.

Maldonado M., Ribes M. and van Duyl F.C. (2012) Nutrient fluxes through sponges: biology, budgets, and ecological implications. *Advances in Marine Biology* 62, 113–182.

Michener R.H. and Kaufman L. (2007) Stable isotope ratios as tracers in marine aquatic food webs: an update. In Michener R.H. and Lajtha K. (eds) *Stable isotopes in ecology and environmental science*. 2nd edition. Oxford: Blackwell Publishing, pp. 238–282.

Mohamed N.M., Colman A.S., Tal Y. and Hill R.T. (2008) Diversity and expression of nitrogen fixation genes in bacterial symbionts of marine sponges. *Environmental Microbiology* 10, 2910–2921.

Moran N.A. (2007) Symbiosis as an adaptive process and source of phenotypic complexity. *Proceedings of the National Academy of Sciences USA* 104, 8627–8633.

Moya A., Peretó J., Gil R. and Latorre A. (2008) Learning how to live together: genomic insights into prokaryote-animal symbioses. *Nature Review Genetics* 9, 218–229.

Muller-Parker G. and Davy S.K. (2001) Temperate and tropical algal-sea anemone symbioses. *Invertebrate Biology* 120, 104–123.

Muscatine L. and Cernichiari E. (1969) Assimilation of photosynthetic products of zooxanthellae by a reef coral. *Biological Bulletin* 137, 506–523.

Muscatine L. and Porter J.W. (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *BioScience* 27, 454–460.

Newsome S.D., Del Rio C.M., Bearhop S. and Phillips D.L. (2007) A niche for isotopic ecology. *Frontiers in Ecology and the Environment* 5, 429–436.

Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'hara R.B., Simpson G.L., Solymos P., Stevens M.H.H. and Wagner H. (2014) *Vegan: community ecology package*. Available at: http://cran.r-project.org/web/packages/vegan.

Roberts D.E., Cummins S.P., Davis A.R. and Pangway C. (1999) Evidence for symbiotic algae in sponges from temperate coastal reefs in New South Wales, Australia. *Memoirs of the Queensland Museum* 44, 493–497.

Ruzicka R. and Gleason D.F. (2009) Sponge community structure and anti-predator defenses on temperate reefs of the South Atlantic Bight. *Journal of Experimental Marine Biology and Ecology* 380, 36–46.

Southwell M.W., Popp B.N. and Martens C.S. (2008) Nitrification controls on fluxes and isotopic composition of nitrate from Florida Keys sponges. *Marine Chemistry* 108, 96–108.

Taylor M.W., Radax R., Steger D. and Wagner M. (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiology and Molecular Biology Reviews* 71, 295–347.

Thacker R.W. and Freeman C.J. (2012) Sponge-microbe symbioses: recent advances and new directions. *Advances in Marine Biology* 62, 57–111.

Thurber A.R. (2007) Diets of Antarctic sponges: links between the pelagic microbial loop and benthic metazoan food web. *Marine Ecology Progress Series* 351, 77–89.

Turner T.F., Collyer M.L. and Krabbenhoft T.J. (2010) A general hypothesis-testing framework for stable isotope ratios in ecological studies. *Ecology* 91, 2227–2233.

Usher K.M. (2008) The ecology and phylogeny of cyanobacterial symbionts in sponges. *Marine Ecology* 29, 178–192.

van Duyl F.C., Moodley L., Nieuwland G., van Ijzerloo L., van Soest R.W.M., Houtekamer M., Meesters E.H. and Middelburg J.J. (2011) Coral cavity sponges depend on reef-derived food resources: stable isotope and fatty acid constraints. *Marine Biology* 158, 1653–1666.

Vrijenhoek R.C. (2010) Genetics and evolution of deep-sea chemosynthetic bacteria and their invertebrate hosts. In Kiel S. (ed.) *The vent and seep biota, Topics in Geobiology* 33. Berlin: Springer, pp. 15–50.

Webster N.S., Negri A.P., Munro M.M. and Battershill C.N. (2004) Diverse microbial communities inhabit Antarctic sponges. *Environmental Microbiology* 6, 288–300.

Weisz J.B. (2006) *Measuring impacts of associated microbial communities on Caribbean reef sponges: searching for symbiosis*. PhD thesis. University of North Carolina at Chapel Hill, North Carolina, USA.

Weisz J.B., Hentschel U., Lindquist N. and Martens C.S. (2007) Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. *Marine Biology* 152, 475–483.

and

Wilkinson C.R. (1983) Net primary productivity in coral reef sponges. *Science* 219, 410–412.

Correspondence should be addressed to:
C.J. Freeman
Smithsonian Marine Station, Fort Pierce, FL, USA
email: freemanc@si.edu

https://doi.org/10.1017/S0025315415000363 Published online by Cambridge University Press