NOTE

Variation in δ^{13}C and δ^{15}N values suggests a coupling of host and symbiont metabolism in the *Symbiodinium–Cassiopea* mutualism

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ABSTRACT: While the 'upside-down' jellyfish *Cassiopea xamachana* feeds heterotrophically, its energy requirements are likely met through its symbiosis with the dinoflagellate symbiont *Symbiodinium*. To investigate the potential coupling of host and symbiont metabolism within *C. xamachana*, we assessed whether variation in the isotope values (δ^{13}C and δ^{15}N) of photosymbiont-rich oral arm tissue was reflected in bell tissue, which is predominately composed of thick animal mesoglea. Samples were collected from 5 geographically disparate sites, including a site influenced by anthropogenically-derived nutrients. Oral arm δ^{13}C values were variable across sites, reflecting varying inputs of marine, terrestrial, and seagrass-derived carbon. Low ($< 0\permil$) δ^{15}N values of oral arm tissue at the 4 non-impacted sites suggests nitrogen derived from nitrogen fixation, while high ($\sim 6\permil$) δ^{15}N values from the impacted site suggests the assimilation of ^{15}N-enriched sources like sewage. Oral arm δ^{13}C and δ^{15}N values accounted for ~75 and 25 % of the variation in bell δ^{13}C and δ^{15}N values. The translocation of symbiont-derived carbon and nitrogen to the host was also supported by evidence of trophic enrichment, with bell tissue enriched on average by 1.7 and 3.4‰ compared to oral arm tissue for δ^{13}C and δ^{15}N, respectively. These data support the contention that microbial symbionts within *Cassiopea* are critical to productivity and nutrient cycling in oligotrophic systems, but also raise important questions about whether symbiont metabolism spurs *Cassiopea* growth and proliferation at sites with chronic anthropogenic nutrient inputs, where higher abundances can have negative effects on local fauna and flora.

KEY WORDS: Symbiosis · Jellyfish · *Cassiopea* · Stable isotopes · Caribbean · Anthropogenic nutrients

INTRODUCTION

Microbial symbionts provide access to sources of nutrients that would otherwise be unavailable to heterotrophic organisms, supporting the proliferation of biodiverse ecosystems in nutrient-poor habitats (Muscatine & Cernichiari 1969). For instance, on oligotrophic reefs photosymbionts and other microbial taxa allow corals and sponges to supplement heterotrophy with inorganic carbon and nitrogen (Muscatine & Porter 1977, Weisz et al. 2010, Freeman et al. 2013, Baker et al. 2015). Research in these systems has historically focused on dominant foundational species, but an increasing number of reports are highlighting the presence and role of these symbionts within other benthic organisms (Verde & McCloskey 1998, Baker 2003, Pitt et al. 2009, Freeman et al. 2016).

Symbiotic scyphozoans like epibenthic jellyfish in the genus *Cassiopea* are abundant in tropical near-shore communities (Pitt et al. 2009). Unlike other jellyfish, they are oriented 'upside-down' to ensure that communities of the dinoflagellate symbiont *Symbiodinium* (Verde & McCloskey 1998, Baker 2003, Coffroth & Santos 2005) within their oral arms (Estes et al. 2003, Welsh et al. 2009, Jantzen et al. 2010, Freeman et al. 2016) are exposed to downwelling sunlight. These photosymbionts are thought to play a dominant role in host carbon and nitrogen metabolism, effectively supplementing heterotrophic feeding on zooplankton and dissolved organic matter (DOM) (Pitt et al. 2009, Welsh et al. 2009, Jantzen et al. 2010, Freeman et al. 2016).

Previous work detailing the symbiont-derived benefit to scyphozoan hosts has been based on one of 2 methods: oxygen and nutrient fluxes in incubation experiments (Verde & McCloskey 1998, Welsh et al. 2009, Jantzen et al. 2010) or the use of isotopically (^{15}N and ^{13}C) labeled compounds (Wilkerson & Kremer 1992, Freeman et al. 2016). While incubation experiments highlight that photosymbionts are capable of fixing more carbon than is needed to compensate for host respiration and elucidate dissolved inorganic nitrogen (DIN) fluxes, this method provides no direct evidence for a translocation of symbiont-derived carbon or nitrogen to the host. By allowing researchers to follow the fate of symbiont-derived nutrients within a symbiosis, isotopic labeling can allow for assessments of nutrient translocation (Freeman et al. 2015). Exposure of the symbiotic scyphozoan *Linuche unguiculata* to $^{15}NH_4$ and subsequent separation of non-algal (containing host cells and small microbes) and zooxanthellae fractions, however, showed that both fractions became enriched in ^{15}N during 2 or 4 h periods of exposure to $^{15}NH_4$ (pulse periods) and provided no evidence for the transfer of ^{15}N-labeled compounds to the host following a 33 h 'chase' period after this initial exposure (Wilkerson & Kremer 1992). Likewise, after incubating *Cassiopea xamachana* in $H^{13}CO_3$ and $^{15}NO_3$ for 6 h, both oral arm (with abundant photosymbiont communities; Verde & McCloskey 1998, Estes et al. 2003) and bell tissue (composed of thick animal mesoglea, with sparse zooxanthellae present only along the epithelial layers; Estes et al. 2003) were enriched in ^{13}C and ^{15}N, but enrichment of both tissues decreased or remained static during an 18 h chase period, countering the idea that nutrient translocation was occurring within the *C. xamachana* holobiont (Freeman et al. 2016). These surprising results may be driven by experiments targeting a metabolic pathway that is carried out by both animal and symbiont

cells or is less efficient than another pathway (e.g. assimilation of NO_3 versus NH_4) (Wilkerson & Kremer 1992, Freeman et al. 2016). Alternatively, the chase period of these studies may not have been long enough to follow the translocation of enriched compounds within the symbiosis.

To overcome the limitations of the studies outlined above, we propose the use of natural abundance stable isotope ratios for carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) to provide a time-integrated estimate of carbon and nitrogen metabolism within *C. xamachana*. $\delta^{13}C$ and $\delta^{15}N$ values are important tools in food web ecology, allowing for estimates of the sources of carbon and nitrogen assimilated by an organism and providing information on the trophic position of consumers within a food web (Michener & Schell 1994). $\delta^{15}N$ values can also be used to trace inputs of anthropogenically-derived nitrogen into coastal ecosystems, with enriched (higher $\delta^{15}N$) values in primary producers and consumers that are chronically exposed to ^{15}N-enriched nutrients derived from sewage (Risk et al. 2009). Instead of isolating zooxanthellae and non-algal fractions from bulk *C. xamachana*, we separated the oral arm and bell tissue (as in Freeman et al. 2016) to allow us to make conclusions regarding the metabolic coupling of oral arm (predominately photosymbiont) and bell (animal cells with some microbes), as would be expected in an obligate mutualism (Freeman et al. 2015). To investigate this coupling, we used the natural variation in the $\delta^{13}C$ and $\delta^{15}N$ values of oral arm and bell tissue collected from 5 geographically disparate sites spanning from tropical Panama to a subtropical site in the Florida Keys, including a site heavily influenced by anthropogenically-derived nutrients (Stoner et al. 2011). We hypothesized that (1) $\delta^{13}C$ and $\delta^{15}N$ values of the oral arm tissue would be variable across sites, reflecting diverse sources of carbon and nitrogen across this latitudinal and anthropogenic gradient, and (2) the $\delta^{13}C$ and $\delta^{15}N$ values of the bell tissue would be tightly coupled with the $\delta^{13}C$ and $\delta^{15}N$ values of the oral arms.

MATERIALS AND METHODS

We collected at least 15 individuals of *Cassiopea xamachana* by snorkeling at 5 sites. Sites included a *Thalassia testudinum* bed in a mangrove channel in the Bocas del Toro archipelago of Panama (see Freeman et al. 2016; herein referred to as Bocas), a *T. testudinum* bed within Twin Cays of Belize (16° 49′ 50.49″ N, 88° 6′ 14.88″ W; herein referred to as Twin Cays), 2 sites in the Bahamas that vary in their prox-

imity to human development and exposure to anthro-
pogenic nutrient inputs (the impacted site Cherokee:
26° 17′ 3.64″ N, 77° 3′ 14.55″ W and the compara-
tively pristine site Snake Cay: 26° 27′ 24.11″ N, 77° 3′
12.88″ W; see Stoner et al. 2011 for site characteris-
tics), and a *T. testudinum* bed near Mote Marine Lab-
oratory in Summerland Key (24° 39′ 38.21″ N, 81° 27′
19.84″ W; herein referred to as Summerland). All
sites were between 2 and 4 m deep. At each site,
small (~8 to 11 cm bell diameter) *C. xamachana* were
collected and placed into a 20 l bucket containing
seawater for transit back to the lab, where they were
placed into flowing seawater until they were sam-
pled. To sample tissues, the manubrium was first sev-
ered to separate the oral arms and bell (as in Free-
man et al. 2016). The tips of several oral arms, where
zooxanthellae abundance is highest and mesoglea
content is low (Estes et al. 2003), were then sampled
and pooled for each individual jellyfish. The portion
of the bell directly underneath the manubrium,
where zooxanthellae abundance and activity is low
(Estes et al. 2003), was excised to obtain bell tissue.
Tissue (bell and oral arm) samples from each individ-
ual were separately bagged and frozen for transport
back to the Smithsonian Marine Station in Fort
Pierce, Florida.

Frozen samples were lyophilized, ground to a fine
powder, dried at 60°C to remove residual moisture,
acidified to remove carbonate by exposure to 12 N
HCl fumes for 12 h, and weighed into silver capsules
as in Freeman et al. (2016). Stable isotope composi-
tions were analyzed at the Stable Isotope Ratio Mass
Spectrometry laboratory at the University of Hong
Kong and are expressed in delta (δ) notation in units
per mil (‰) (Fry 2006). Precision of $\delta^{13}C$ and $\delta^{15}N$
samples was 0.02 and 0.6, respectively, as determined
by analysis of the internal acetanilide standard ('acet
6'; 70% C). The organic matter content of some sam-
ples was too low (Pitt et al. 2009) for accurate $\delta^{13}C$ and
$\delta^{15}N$ values. These data were not included in future
analyses, leading in some cases to final sample sizes
of less than 15 (see Table 1 for sample sizes).

To quantify the relative effects of collection site and
tissue type (bell or oral arm) on the $\delta^{13}C$ and $\delta^{15}N$ val-
ues of individual samples, we measured the isotopic
dissimilarity between samples (as Euclidean dis-
tance), created a distance matrix of all data points,
and analyzed sample distances using a permuta-
tional analysis of variance (PERMANOVA) with the R
function 'adonis' in the package 'vegan' (Oksanen et
al. 2017). To examine the relationship between the
isotope values of bell and oral arm tissue, we used
linear regressions in Systat (v.11).

RESULTS

The isotope values ($\delta^{13}C$ and $\delta^{15}N$) of oral arm tis-
sue were variable between the 5 sites, with mean
$\delta^{13}C$ values ranging from −18.6‰ at Bocas to −15.5‰
in Twin Cays, and $\delta^{15}N$ values ranging from −1.4‰ at
Snake Cay to 5.98‰ at Cherokee (Fig. 1); a similar
trend was observed with bell tissue (mean $\delta^{13}C$ val-
ues ranging from −17.02‰ at Bocas to −13.6‰ in
Twin Cays and $\delta^{15}N$ values ranging from 2.2‰ at
Summerland to 10.35‰ at Cherokee; Fig. 1). Varia-
tion in $\delta^{13}C$ and $\delta^{15}N$ values was strongly influenced
by site, with over 75% of the variation in isotopic dis-
similarity between samples being attributed to col-
lection site (PERMANOVA on effect of collection
site: $r^2 = 0.78$, $p = 0.001$). When data from each tissue
type were analyzed separately, this trend was even
stronger, with over 90% of the variation in the iso-
topic dissimilarity between individual samples being
attributed to collection site (PERMANOVA on effect
of collection site: $r^2 = 0.92$, $p = 0.001$ both for bell and
oral arm samples). Tissue type (oral arm or bell) also
explained a significant portion of the variation in iso-
topic dissimilarity between individual samples (PERM-
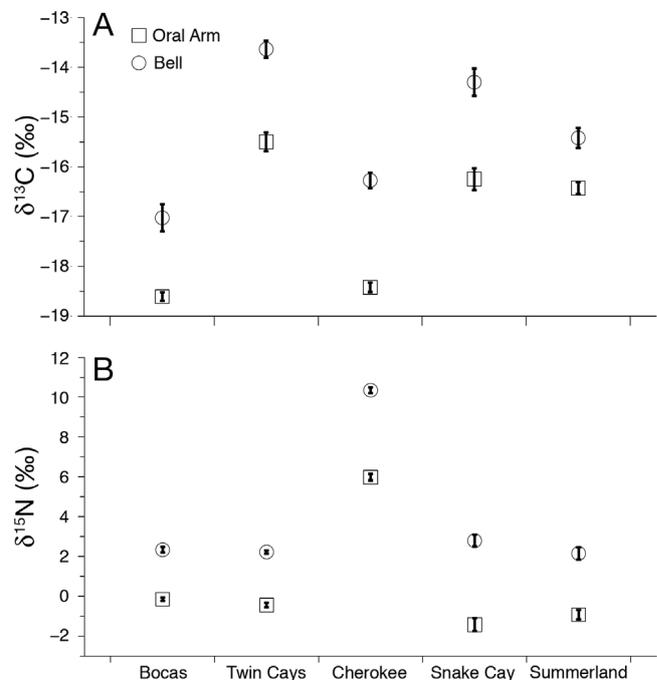ANOVA on tissue type: $r^2 = 0.25$, $p = 0.001$) and

Fig. 1. Mean (±SE) (A) $\delta^{13}C$ and (B) $\delta^{15}N$ values of *Cassiopea
xamachana* bell (circle) and oral arm (square) tissue from 5
sites across the Caribbean and Florida Keys. Sample size (N)
of bell/oral arm tissue: 8/14 for Bocas, 16/16 for Twin Cays,
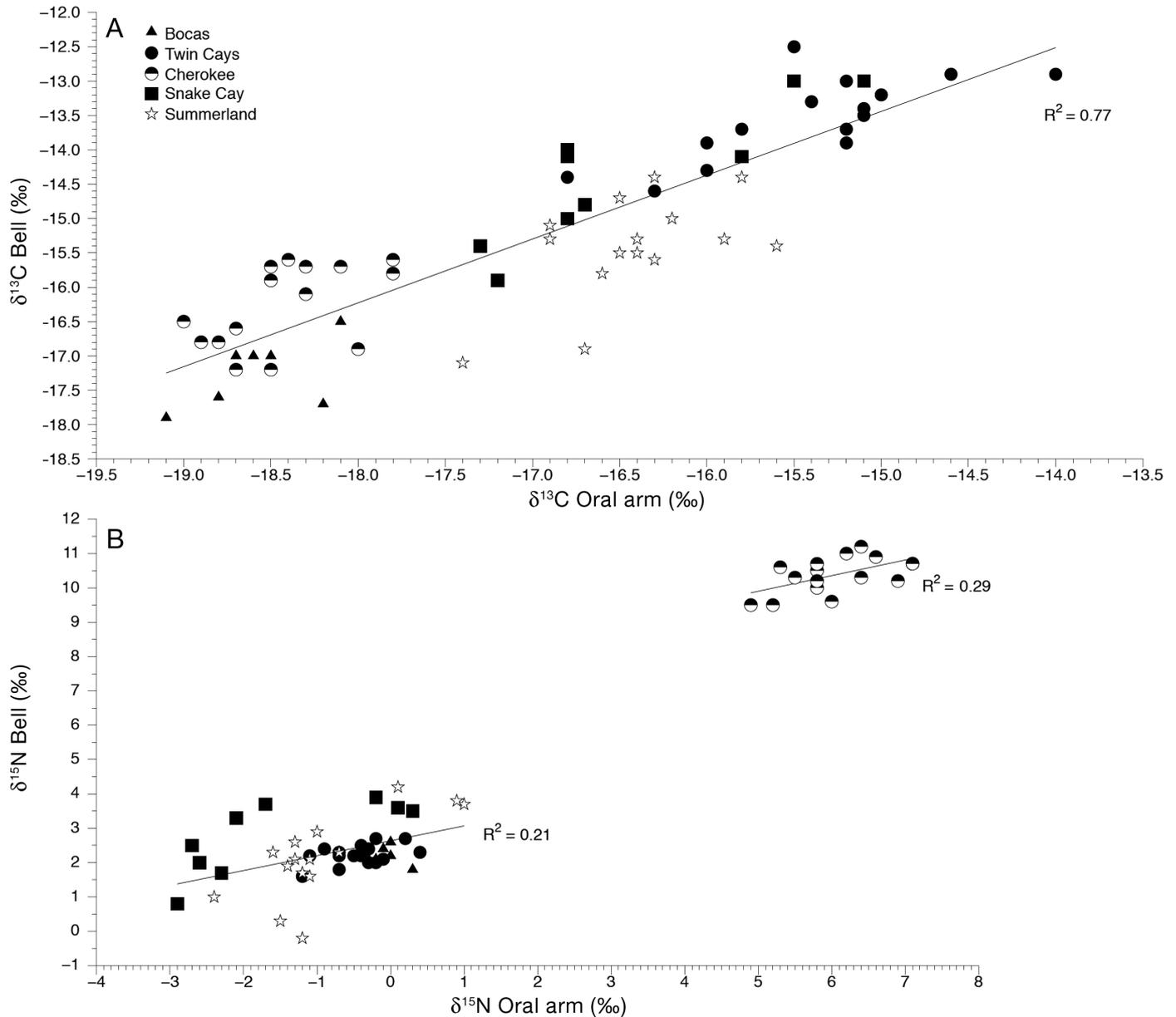15/15 for Cherokee, 11/13 for Snake Cay, and 15/15 for
Summerland

Fig. 2. (A) $\delta^{13}C$ and (B) $\delta^{15}N$ values of *Cassiopea xamachana* bell tissue as a function of the $\delta^{13}C$ or $\delta^{15}N$ values of corresponding oral arm tissue from 5 sites across the Caribbean and Florida Keys. Sample size (N): 7 for Bocas, 16 for Twin Cays, 15 for Cherokee, 9 for Snake Cay, and 15 for Summerland

together tissue type and site accounted for over 90% of the variation in $\delta^{13}C$ and $\delta^{15}N$ values of individual samples (PERMANOVA: $r^2 = 0.93$).

There was a significant relationship between the $\delta^{13}C$ of bell and oral arm tissue from all sites (linear regression: $r^2 = 0.77$, $p < 0.001$; Fig. 2a) and also within individual sites, except at Bocas (Table 1). Although there was also a strong relationship between the $\delta^{15}N$ values of bell and oral arm tissue (linear regression: $r^2 = 0.931$, $p < 0.001$), this trend was strongly driven by the enriched $\delta^{15}N$ values of sam-

ples collected at Cherokee. When individuals from Cherokee were analyzed separately, these trends were still present, but were weaker (linear regression: $r^2 = 0.29$, $p < 0.05$ and $r^2 = 0.21$, $p < 0.01$ for Cherokee alone and all sites except Cherokee, respectively; Fig. 2b). The relationship between the $\delta^{15}N$ values of bell and oral arm tissue was significant at Cherokee, Snake Cay, and Summerland (Table 1).

$\delta^{13}C$ and $\delta^{15}N$ values of bell tissue were consistently enriched compared to oral arm tissue (Fig. 1), with an average enrichment across all samples of 1.7

Table 1. Linear regressions comparing $\delta^{13}C$ and $\delta^{15}N$ values of bell and oral arm tissue within *Cassiopea xamachana* across 5 sites spanning from Panama (Bocas) to the Florida Keys (Summerland). Also shown is the mean (±SE) $\delta^{13}C$ and $\delta^{15}N$ enrichment of bell tissue relative to oral arm tissue within a site. Sample sizes for these comparisons are shown for reference. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Site	N	$\delta^{13}C$ bell–oral arm r^2	$\delta^{15}N$ bell–oral arm r^2	$\delta^{13}C$ difference (± SE)	$\delta^{15}N$ difference (± SE)
Bocas	7	0.32	0.16	1.36 (0.16)	2.33 (0.15)
Twin Cays	16	0.65***	0.22	1.86 (0.12)	2.66 (0.10)
Cherokee	15	0.28*	0.29*	2.14 (0.13)	4.38 (0.15)
Snake Cay	9	0.76**	0.58*	2.09 (0.16)	4.36 (0.28)
Summerland	15	0.32*	0.54**	1.02 (0.16)	3.08 (0.22)

and 3.4‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, and a range of bell $\delta^{13}C$ enrichment from 1.02‰ at Summerland to 2.14‰ at Cherokee, and bell $\delta^{15}N$ enrichment from 2.3‰ at Bocas to 4.4‰ at Cherokee and Snake Cay (Table 1).

DISCUSSION

Symbiotic scyphozoans couple heterotrophic feeding on zooplankton and particulate or DOM (Wilkerson & Kremer 1992, Pitt et al. 2009, Welsh et al. 2009) with the metabolism of photosymbionts like *Symbiodinium* (LaJeunesse 2002) and other microbial groups (Pitt et al. 2009, Jantzen et al. 2010, Freeman et al. 2016). Our results support the contention that *Cassiopea xamachana* rely on the metabolic products of *Symbiodinium* communities within their oral arms. $\delta^{13}C$ and $\delta^{15}N$ values in the current study also suggest that *C. xamachana* holobionts (host and symbiont) assimilate local sources of carbon and nitrogen and are capable of utilizing both natural and anthropogenic nutrient sources.

Abundant photosymbiont communities in the oral arms of *Cassiopea* jellyfish (Verde & McCloskey 1998, Estes et al. 2003, Freeman et al. 2016) allow for the assimilation of dissolved organic or inorganic carbon (DOC/DIC), leading to $\delta^{13}C$ values reflective of carbon sources that are supporting local food webs. Sites were generally delineated into 2 main groups based on their $\delta^{13}C$ values, with depleted $\delta^{13}C$ values at Cherokee and Bocas and more enriched $\delta^{13}C$ values at Twin Cays, Snake Cay, and Summerland. Depleted $\delta^{13}C$ values at Cherokee are likely driven by the close proximity of these jellyfish to human development (there are 165 structures adjacent to this site, with jellyfish collected in the harbor ~50 m from the nearest building) and chronic inputs of anthropogeni-

cally-derived nutrients (Lamb & Swart 2008, Stoner et al. 2011). Although the Bocas del Toro archipelago as a whole is characterized by high inputs of allochthonous organic carbon from agriculture, with some areas heavily impacted by anthropogenically-derived nutrients (D'Croz et al. 2005, Aronson et al. 2014), our collection site for *C. xamachana* was ~6 km from dense human development in Bocas town and Bastimentos. Therefore, all 4 of the other sites are likely to be less impacted by chronic disturbances from large-scale human development than Cherokee. Oral arm $\delta^{13}C$ values at these other sites likely reflect local variation in the sources of DOC or DIC. For instance, fractionation during carbon fixation leads to depleted $\delta^{13}C$ values of –20 to –30‰ in mangroves and other C3 plants (Fry 2006, Lamb & Swart 2008), while more enriched $\delta^{13}C$ values are found in seagrass (–5 to –13‰; Fourqurean et al. 2005) and carbon from marine plankton (–15 to –22‰; Lamb & Swart 2008). Thus, symbionts within the oral arms of jellyfish from the mangrove channel in Bocas may be assimilating more of their carbon from DOC and DIC derived from nearby C3 plants than symbionts within *Cassiopea* from sites like Twin Cays, Snake Cay, and Summerland that have direct connections to the ocean and are therefore influenced more by marine sources of carbon.

Oral arm $\delta^{15}N$ values at or below 0‰ at all sites except Cherokee suggest that symbionts within *C. xamachana* are assimilating inorganic nitrogen derived from N-fixation at non-impacted sites (Fry 2006). Alternatively, the microbiomes of *C. xamachana* may include symbionts capable of N-fixation, as has been reported in corals (Lesser et al. 2004) and sponges (Mohamed et al. 2008). Additional work is needed to elucidate the structure and function of these microbial communities within symbiotic scyphozoans (Pitt et al. 2009, Welsh et al. 2009, Freeman et al. 2016). The striking ^{15}N enrichment of *C. xamachana* oral arm tissue from Cherokee (>6‰ relative to the other 4 sites) suggests that these jellyfish predominately assimilate anthropogenically-derived nutrients from ^{15}N-enriched sources like sewage (Heikoop et al. 2000). This site is heavily impacted, with NH_4 concentrations in the water of 8.5 µM (Stoner et al. 2011) compared to Snake Cay (NH_4: 1.91 µM; Stoner et al. 2011), Twin Cays (NH_4: 0.38 to 1.40 µM; Lapointe et al. 1987), the Lower Florida Keys (NH_4: 0.4 to 1.4 µM; Lapointe & Clark 1992, Lamb & Swart 2008), and Bocas del Toro, Panama (NH_4: 0.20 to 0.26 µM; Carruthers et al. 2005).

Together, our isotope data suggest that *C. xamachana* integrates the signal from local sources of natural and anthropogenically-derived nutrients, acting as a record of spatial variation in baseline $\delta^{13}C$ and $\delta^{15}N$ values. With their rapid tissue $\delta^{13}C$ and $\delta^{15}N$ turnover rates, widespread distribution, and ease of sampling, jellyfish may therefore be useful sentinel organisms for monitoring programs in coastal ecosystem (MacKenzie et al. 2014, D'Ambra et al. 2014).

Bell $\delta^{13}C$ values closely mirrored the $\delta^{13}C$ values of the corresponding oral arm, providing evidence for a metabolic coupling of these tissues and supporting the assertion that host cells derive carbon from photosymbiont metabolism in the oral arms (Pitt et al. 2009, Freeman et al. 2015, 2016). In contrast, if host cells in the bell were deriving a predominant portion of their carbon from heterotrophic feeding at higher trophic levels (Pitt et al. 2009), we would expect more of a metabolic decoupling between oral arm and bell tissue $\delta^{13}C$ values. Although the relationship between bell and oral arm $\delta^{15}N$ values was significant, oral arm $\delta^{15}N$ explained less than 30% of the variation in bell values. It is likely that bell nitrogen was therefore derived from a combination of DIN from photosymbionts in the oral arms, exogenous sources via heterotrophy, and microbial metabolism in the bell (Pitt et al. 2009, Freeman et al. 2016). Because $\delta^{13}C$ and $\delta^{15}N$ values typically increase with each trophic level, the mean enrichment of bell tissue relative to oral arm tissue by 1.7 and 3.4‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, supports our conclusion that these trends are driven by nutrient translocation. This trophic enrichment is generally assumed to be ~1 and 3.4‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, but species-specific metabolic processes can lead to substantial variation in these values (McCutchan et al. 2003, Fry 2006). The unique physiology of gelatinous zooplankton like jellyfish, in particular, can influence this trophic enrichment, with values of 4 and 0.1‰ reported for enrichment in $\delta^{13}C$ and $\delta^{15}N$ values, respectively (D'Ambra et al. 2014). Although controlled laboratory studies similar to D'Ambra et al. (2014) have not been conducted for *Cassiopea* spp., enriched $\delta^{13}C$ and $\delta^{15}N$ values found in the bell tissue of the current study are suggestive of a trophic step between these 2 tissue types, providing complementary data that nutrient translocation is occurring. Interestingly, there was variation in both the strength of carbon and nitrogen coupling and the relative enrichment of bell tissue across these 5 sites, suggesting that environmental conditions may influence these interactions. Future research investigating the reliance of symbiotic scyphozoans on autotrophically- or het-

erotrophically-derived nutrients across environmental gradients or in manipulative experiments (Freeman & Thacker 2011) may help to elucidate this shift.

In conclusion, we present novel evidence supporting the role of microbial symbionts in *Cassiopea* metabolism within both pristine and impacted coastal habitats. With a recent report showing that the efficiency of symbiont carbon and nitrogen assimilation in *C. xamachana* is comparable to, or exceeds, that of soft corals and sponges (Freeman et al. 2016), the ability to fix nutrients at oligotrophic sites may have positive effects on local productivity and nutrient cycling (Pitt et al. 2009). The fixation of inorganic nutrients by *C. xamachana* may also make these nutrients available to consumers, providing an important link in the local food webs (Brandon & Cutress 1985, Stoner & Layman 2015). Symbiont metabolism may, however, lead to the proliferation of *Cassiopea* at impacted sites like Cherokee (Stoner et al. 2011, Stoner et al. 2016), with abundant populations of *Cassiopea* driving down seagrass cover and shoot density and having a negative effect on benthic faunal density (Stoner et al. 2014). The data presented here support the contention that jellyfish are an important, trophically diverse group that should be included in ecosystem models (Fleming et al. 2015), and that future work investigating the interaction between this symbiosis and the environment is warranted.

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