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Mesophotic corals in Hawai'i maintain autotrophy to survive low-light conditions

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In mesophotic coral ecosystems, reef-building corals and their photosynthetic symbionts can survive with less than 1% of surface irradiance. How depth-specialist corals rely upon autotrophically and heterotrophically derived energy sources across the mesophotic zone remains unclear. We analysed the stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values of a *Leptoseris* community from the 'Au'au Channel, Maui, Hawai'i (65–125 m) including four coral host species living symbiotically with three algal haplotypes. We characterized the isotope values of hosts and symbionts across species and depth to compare trophic strategies. Symbiont $\delta^{13}\text{C}$ was consistently 0.5‰ higher than host $\delta^{13}\text{C}$ at all depths. Mean colony host and symbiont $\delta^{15}\text{N}$ differed by up to 3.7‰ at shallow depths and converged at deeper depths. These results suggest that both heterotrophy and autotrophy remained integral to colony survival across depth. The increasing similarity between host and symbiont $\delta^{15}\text{N}$ at deeper depths suggests that nitrogen is more efficiently shared between mesophotic coral hosts and their algal symbionts to sustain autotrophy. Isotopic trends across depth did not generally vary by host species or algal haplotype, suggesting that photosynthesis remains essential to *Leptoseris* survival and growth despite low light availability in the mesophotic zone.

1. Introduction

For photosynthetic organisms, resource competition theory suggests that niches are differentiated by the use of limiting resources, such as nutrients and light, across a heterogeneous habitat [1,2]. Niche differentiation in reef corals can be driven by species-specific patterns in depth zonation [3], colony morphology [4,5] and nutrient acquisition [6–8]. Most shallow reef-forming corals depend on photosynthetic algal endosymbionts (family Symbiodiniaceae [9]) for energy from autotrophy [10]. To acquire nutrients, corals can also consume seston heterotrophically, including detritus and zooplankton, through oral openings in modular units called polyps [6,11,12]. The mutualisms between corals and their algal symbionts can develop distinct, mixotrophic niches in reef ecosystems defined by reliance on autotrophic versus heterotrophic energy sources [6]. These niches enable reef-building corals to construct the most biologically diverse marine ecosystems on the planet, supporting tens of millions of people worldwide with food, coastal protection and other ecosystem services [13].

Comparisons of stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes between host tissue and algal symbionts have been used to assess coral trophic strategies [3,4,12,14,15]. For corals reliant on autotrophy, host $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ closely follow those of the symbionts because carbon and nitrogen are translocated

to the host following photosynthesis [3,12,16]. During heterotrophy, corals may rely on seston more than symbiont photosynthates [17]. When seston isotopic values differ from Symbiodiniaceae values, increases in heterotrophy and decreases in autotrophy cause host $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to deviate from symbiont values [3,6,12,14,15,18,19]. Muscatine *et al.* [12] reported increasing differences between host and symbiont $\delta^{13}\text{C}$ with depth (1–50 m), hypothesizing that, as depth increases, symbiotic corals rely relatively less on autotrophy and more on heterotrophy for carbon. However, little is known about how trophic dynamics vary among communities of depth-specialist corals, particularly at depths greater than 60 m [3,7].

Mesophotic coral ecosystems are characterized by communities of corals and other organisms living from approximately 30 m to more than 150 m in depth [20]. Despite limited light availability and changing spectral composition with depth, photosynthetic corals are crucial members of the mesophotic community [15], surviving with less than 1% of surface irradiance [3,21]. Mesophotic corals have adapted to form vast reefs at low light levels through flattened colony morphologies [4,21,22], specialized skeletal micro-morphologies [5,23,24] and pigment concentrations [3,25]. While these photoadaptations may enhance autotrophy in mesophotic environments, numerous studies have hypothesized that mesophotic corals adjust trophic strategies between autotrophy and heterotrophy to inhabit deeper depths [3,7,8,15,21,26,27]. However, the extent that mesophotic reef corals rely on heterotrophy versus autotrophy with increasing depth is debated [16,27,28]. Recent evidence of anthropogenic impacts on mesophotic reefs, including elevated temperatures [29] and nutrient pollution [30], emphasize the need to conserve these vast ecosystems. Effective management strategies will require a better understanding of energy uptake, allocation and niche differentiation in mesophotic corals.

Clarifying the relative trophic contributions to corals at mesophotic depths could explain how they survive in light-limited environments. Most studies characterizing trophic dynamics in mesophotic corals have described their heterotrophic and autotrophic capacity relative to shallow reef counterparts [4,5,7,8,12,15]. While most isotopic studies support that heterotrophy supplants autotrophy in corals at increasing depths [27], variability in host–symbiont carbon exchange and symbiont type can obscure trophic trends across depth and light gradients [3,16,28]. Isotopic studies reveal conflicting trends across mesophotic depths, suggesting that some Caribbean and Red Sea corals may not increase rates of heterotrophy or reduce reliance on autotrophy from 5 m to at least 60 m [4,14,31]. $\delta^{15}\text{N}$ values can decrease with increasing depth for both hosts and symbionts assessed separately [11], contrary to trophic increases in host $\delta^{15}\text{N}$ expected under increased heterotrophy [6]. Some studies reported no change in host $\delta^{15}\text{N}$ and symbiont $\delta^{15}\text{N}$ across the mesophotic zone [4,15]. Prior studies have identified several hypotheses to explain changes in $\delta^{15}\text{N}$ with depth. Variations in light quality/availability [32,33], nitrogenous waste exchange between host and symbiont cells [4,15,19], and nitrogen-specific rates of symbiont growth [11] impact the retention or excretion of ^{15}N and ^{14}N and resulting $\delta^{15}\text{N}$ values. However, it remains unclear how the acquisition and/or exchange of nitrogen and carbon contribute to the trophic strategies of mesophotic corals.

Assessing trophic status across mesophotic depths is complicated by various genotypic combinations of host species and algal symbionts. Depth zonation within mesophotic coral communities occurs based on host species [23,34,35] and algal symbiont diversity [8,25,35]. While some coral species maintain one haplotype of algal symbionts across mesophotic depths [25], others associate with multiple haplotypes across depths [3,35]. Variable combinations of host species and symbiont haplotypes may create divergent photophysiological and trophic strategies across the mesophotic zone.

The Hawaiian Archipelago's *Leptoseris* communities create the most spatially extensive mesophotic coral ecosystems on record, dominating from 60 to 160 m [36,37] with up to 100% live coral cover at some depths [34,38]. Phylogenetic analyses of the *Leptoseris* community (65–150 m) have identified six host species (*L. hawaiiensis*, *L. yabei*, *L. papyracea*, *L. scabra*, *L. tubulifera* and *Leptoseris* sp. 1) [23,35] and three algal haplotypes of the genus *Cladocopium* [35]. Depth partitioning of *Leptoseris* species has been linked to different photoacclimatization strategies including increases in chlorophyll *a*, symbiont density and dark-adapted yield in species persisting through deeper depths, which could sustain autotrophy at depth [3].

We investigated how trophic strategies influence niche partitioning across depth in a *Leptoseris* mesophotic reef community in the 'Au'au Channel off Maui, Hawai'i (65–125 m). We analysed mesophotic *Leptoseris* colonies representing four host species living symbiotically with three algal haplotypes ([35]; electronic supplementary material, figure S1A). The six combinations of host species and algal haplotypes varied in distribution across depth ([35]; electronic supplementary material, figure S1B). While Padilla-Gamiño *et al.* [3] reported stable isotope values from a *Leptoseris* sample set collected from the 'Au'au Channel in 2009–2010, the coral and algal genotypes were unknown. Here, we measured the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of hosts and symbionts across genotype and depth collected from a separate cruise in February–March 2011 to assess differences in trophic strategies among *Leptoseris* species.

2. Methods

(a) Colony collection and genetic identification

Fragments of cryptic *Leptoseris* spp. colonies ($n = 47$) were collected from depths of 65–125 m from the 'Au'au Channel offshore of Olowalu, West Maui from 27 February to 3 March 2011 by submersible (*Pisces IV* and *Pisces V*). Irradiance was approximately $5\text{--}75\ \mu\text{mol photons m}^{-2}\text{ s}^{-1}$ [3]. Collections are described in Pochon *et al.* [35] and Padilla-Gamiño *et al.* [3]. Briefly, coral fragments (approx. 5–8 cm plates) were collected haphazardly from the centre of *Leptoseris* reefs across depth; colonies at the same depth were interspaced by at least 10 m. Collected samples were processed and frozen at -80°C within 3–9 h of ascent aboard the R/V Ka'imikai-O-Kanaloa. *Leptoseris* colonies analysed in this study were identified by host species and symbiont haplotype in Pochon *et al.* [35]. Pochon *et al.* identified each colony by host species using COX1–1-rRNA introns and by symbiont haplotype using algal symbiont COI mitochondrial markers, distinguished by ITS2 community sequence profiles [3,35]. We referred to COI sequence types because we lacked the samples required to reanalyse colony fragments for individual ITS profiles. Since COI sequence types represented distinct genetic groups of symbionts (i.e. haplotypes, with

some overlap in *ITS2* assemblages) within *Cladocopium* [35], we labelled colonies by COI haplotype 1–3. We studied the subset of the colonies in Pochon *et al.* [35] with remaining tissue available for stable isotope analyses.

As presented in Pochon *et al.* [35], our samples represent four *Leptoseris* host species (*L. hawaiiensis*, *L. scabra*, *L. tubulifera* and *Leptoseris* sp. 1) and all three algal haplotypes (electronic supplementary material, figure S1A). All colonies hosted only one algal haplotype; several *Leptoseris* species can host the same algal haplotype and different colonies of the same species can host different algal haplotypes [35]. Eleven colonies were *L. scabra* (65–99 m); ten hosted haplotype COI-2 and one hosted COI-3 (electronic supplementary material, figure S1B). Eleven colonies were *L. tubulifera* (73–99 m), hosting haplotype COI-2. Fourteen *Leptoseris* sp. 1 colonies were found across the widest depth range (67–123 m); five hosted haplotype COI-2 (67–99 m) and nine hosted COI-3 (67–123 m). Eleven *L. hawaiiensis* were found only at the deepest depths of 123–125 m, all hosting haplotype COI-1. Notably, all *L. hawaiiensis* and one *Leptoseris* sp. 1 colony were collected past the depth of 1% surface irradiance [3] (112 m; electronic supplementary material, figure S1B), traditionally considered the depth limit of photosynthetic corals [21].

(b) Stable isotopic analyses

For each fragment (approx. 5–8 cm), coral tissue was removed from the skeleton using a pressurized airbrush and deionized water spray. We separated each colony into host and symbiont fractions prior to isotopic analyses [3,4,12,14,15]. The slurry was ground in a tissue homogenizer and centrifuged at 2880 relative centrifugal force (RCF) for 10 min to separate host and symbiont cells into distinct layers [39]. Residual skeletal material pelleted into its own layer below the symbionts during centrifugation. Routine microscope checks of symbiont samples confirmed a lack of skeletal contamination in the symbiont cells; to avoid altering the isotopic signatures, we did not acidify samples [40]. Host and symbiont fractions were separated by microspatula and pipetted into pre-weighed tin capsules, dried in an oven at 60°C for at least 24 h [41], and reweighed. All capsules were folded into compact cubes and combusted using an Elementar Pyrocube elemental analyser; resulting CO₂ and N₂ gases were analysed using an Elementar Isoprime100 isotope ratio mass spectrometer. Host and symbiont $\delta^{13}\text{C}$ values were reported relative to Vienna Pee Dee belemnite (vPDB) limestone standard ($\delta^{13}\text{C}$ = per mil deviation of the ratio of stable carbon $^{13}\text{C}:^{12}\text{C}$ relative to vPDB) and $\delta^{15}\text{N}$ values were reported relative to air ($\delta^{15}\text{N}$ = per mil deviation of the ratio of stable nitrogen $^{15}\text{N}:^{14}\text{N}$ relative to air). Standards were analysed, including B2150 (EA Consumables, LLC, Marlton, NJ, USA), internal elk tissue, dogfish muscle reference material (DORM), and bird feathers, with a precision of $\pm 0.1\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$. Approximately 21% of host and symbiont samples ($n = 10$ each) were analysed in duplicate.

(c) Statistical analysis

For statistical analyses of the *Leptoseris* community, similar to Padilla-Gamiño *et al.* [3], we included all *Leptoseris* colonies across the collection depth range (65–125 m). Our sampling location enabled us to collect *L. hawaiiensis* only from 123 to 125 m; *L. hawaiiensis* was the only species collected past 100 m except for one *Leptoseris* sp. 1 colony. Although *L. hawaiiensis* is regarded as a depth specialist in the lower mesophotic zone [3,35], its depth range (80–130 m; [23]) overlaps with the other species. In addition, while our *L. hawaiiensis* were found exclusively with haplotype COI-1, other *Leptoseris* colonies containing haplotype COI-1 have been identified at depths as shallow as 95 m [35]. Since the known habitat range of *L. hawaiiensis* and its algal haplotype overlap with the other

study species, we included it in all community assessments and statistically tested its influence on community trends.

To identify outliers, histogram distributions of all $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were plotted by fraction type (host or symbiont) in JMP Pro 14; residuals and Cook's distances were used to confirm outliers (electronic supplementary material, table S1). We excluded outlier values that exceeded the interquartile range by more than ± 1.5 times ($n =$ one $\delta^{13}\text{C}$ from a host sample; one $\delta^{13}\text{C}$ from a symbiont sample; two $\delta^{15}\text{N}$ from host samples and two $\delta^{15}\text{N}$ from symbiont samples were excluded).

To initially assess the trophic relationship between hosts and symbionts across the *Leptoseris* community, we compared $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values using the Stable Isotope Bayesian Ellipses in R (SIBER) package (R version 4.0.2) [42]. SIBER visualizes host and symbiont isotopic niches to determine the trophic niche overlap (i.e. extent of symbiotic resource sharing) between coral hosts and their algal symbionts [6,27]. Since SIBER can only effectively compare groups with the same or different sample sizes of at least 20 samples each [43], we pooled samples ($n = 47$ colonies) at the genus level [6] and used SIBER to assess broad trophic trends across the community. We generated 4000 standard ellipse areas corrected for sample size (SEA_C) with the SEA_C encompassing 40% of the dataset variation [6]. We conducted a residual permutation procedure of 100 000 permutations of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to verify isotopic differences between the centroids of host and symbiont datasets [6,27,44].

We compared ellipse overlap for all *Leptoseris* colonies against a proposed spectrum of coral trophic strategies using percentage cut-offs for heterotrophy (less than or equal to 10% overlap), mixotrophy (10–70% overlap) and autotrophy (greater than or equal to 70% overlap) [6]. We calculated mean per cent overlap between host and symbiont ellipses, with per cent overlap defined as the overlapping isotopic area divided by the host ellipse area [6,27]. To evaluate the potential effect of collection depth on *Leptoseris* host and symbiont isotope values, we replotted values pooled into 5 m depth bins. Sample sizes among host species and symbiont haplotypes (electronic supplementary material, figure S2) were insufficient to analyse independently by SIBER.

To investigate trophic relationships among *Leptoseris* host species and symbiont haplotypes across depth, we conducted simple linear regressions in JMP Pro 14. To investigate niche partitioning in *Leptoseris* colonies across depth, we tested for significant linear relationships between isotope values and depth for each host species or symbiont haplotype. We tested for linear relationships of host $\delta^{13}\text{C}$, symbiont $\delta^{13}\text{C}$ and $\delta^{13}\text{C}_{\text{host-symbiont}}$ versus depth by host species and symbiont haplotype. We repeated the same analyses for host $\delta^{15}\text{N}$, symbiont $\delta^{15}\text{N}$ and $\delta^{15}\text{N}_{\text{host-symbiont}}$ versus depth (electronic supplementary material, table S2). Sample distributions were checked for normality and homoscedasticity when tested for statistical significance at the two-sided $\alpha = 0.05$ level. To test the influence of *L. hawaiiensis* on community trends, we repeated community-wide regressions excluding *L. hawaiiensis* (electronic supplementary material, table S3).

We compared the slopes of significant regressions across depth with one another by host species and algal haplotype using a multi-variable regression analysis. To determine whether there was a significant difference in the slope of isotope values based on host species or symbiont haplotype, each model included an interaction term between collection depth and either host species or symbiont haplotype. The error rate for the two-sided p -value for the interaction term was $\alpha = 0.10$ accounting for multiple analyses on the same dataset. When interaction term $p < 0.10$, the model was used to determine whether there were significant differences among regression slopes (two-sided $\alpha = 0.05$) based on colony host species or algal haplotype (electronic supplementary material, table S2). The same multi-variable regression analysis was applied to the

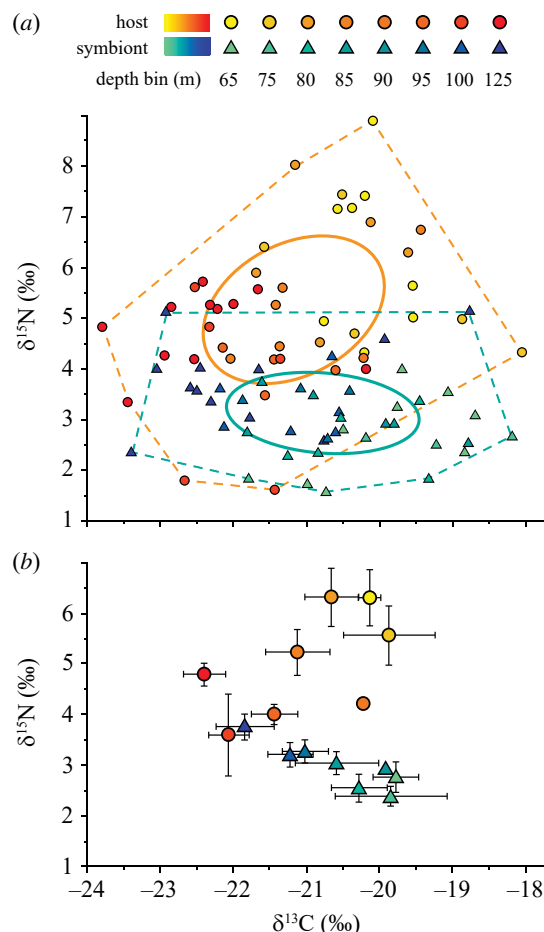


Figure 1. Heterotrophy plays a dominant role in *Leptoseris* nutrition, but host and symbiont stable isotope values converge across mesophotic depths. (a) SIBER model outputs for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ values of all samples with dotted lines denoting convex hulls of hosts (orange) and symbionts (teal). Solid rings of the same colours denote standard ellipse areas corrected for sample size (SEA_C). (b) Isotopic values for hosts and symbionts pooled by 5 m collection depth bins (means $\pm$ s.e.; electronic supplementary material, table S4).

community dataset excluding *L. hawaiiensis* to determine whether this deeper species had a notable effect on community trends (electronic supplementary material, table S3).

3. Results and discussion

(a) Heterotrophy plays a dominant role in mesophotic coral nutrition

For the *Leptoseris* community, host and symbiont isotopic niche spaces were significantly different from one another (residual permutation procedure $p < 0.001$), with 3.63% of the SIBER host ellipses overlapping the symbiont ellipses (figure 1a). A host–symbiont ellipse overlap less than or equal to 10% of the host SEA_C suggests a heterotrophy-dominant strategy for this community [6], supporting that heterotrophy is essential to mesophotic reef-building corals including *Leptoseris* [3,4,7,8,31]. While heterotrophy is an important energy source for mesophotic corals, deep-water *Leptoseris* have been found with only vestigial tentacles that cannot extend [24,45]. These corals might obtain heterotrophic nutrients from the environment by moving their vestigial tentacles to circulate organic matter into their polyps. A similar mechanism occurs in *Leptoseris fragilis*,

which lacks tentacles [21,26] and instead pumps water through the mouth and out of epidermal pores. This mechanism generates a flow-through filtration system for consuming particulate organic matter (POM) and dissolved organic matter (DOM) like a sponge [26]. Coral species such as *Mycectophyllia reesi*, which lacks tentacles, capture food particles with mucus secretions [46]. Mucus channels can regulate shared feeding across colony polyps [47] and may facilitate feeding in *Leptoseris* corals. While the plating morphology of *Leptoseris* is probably adapted to optimize light capture at depth [11,14], plating may enhance passive feeding on sinking particles of detritus that collect on colony surfaces [7].

While SIBER suggests that heterotrophy is a major energy source for *Leptoseris* corals, various factors could have obscured the contribution of autotrophy to colony survival and growth. Our colonies were collected during the reduced seasonal photoperiods of winter–early spring. As observed in temperate symbiotic corals with seasonal variation in irradiance and photosynthetic rate, corals in low irradiance can still rely on autotrophic carbon for metabolic energy even when irradiance is insufficient to sustain host growth [17]. Additionally, SIBER trophic strategy classifications were based on shallow reef corals living at a maximum depth of 5 m [6] where up to 90% of carbon from photosynthates may be translocated to the coral host [48]. At 5 m, trophic niche overlaps of up to 94% indicate a high degree of resource sharing through photosynthesis [6]. Photosynthetic rates that facilitate coral growth at shallow depths are unlikely in low-irradiance mesophotic environments. Red Sea *Leptoseris* colonies (82–90 m) collected in autumn exhibited photosynthetic rates up to 10 times lower than most shallow (10 m) symbiotic corals from the same reefs [7]. Photosynthetic rates were too low to sustain daily respiration rates, which were supplemented by heterotrophy [7]. Trophic classifications established by SIBER may not account for photosynthetic contributions to metabolism required for growth, or how autotrophic contributions to growth and metabolism vary in the mesophotic zone [7,16,24,28].

Grouping by 5 m collection depth bins indicates that $\delta^{13}\text{C}$ values for both fractions decreased with depth (65–125 m), with the mean difference between host and symbiont fractions varying by 0.3–0.9‰ (figure 1b; electronic supplementary material, table S4). Host and symbiont $\delta^{15}\text{N}$ converged with deeper depths, as the mean difference between host and symbiont decreased by 2.5‰ from 65 to 125 m (figure 1b; electronic supplementary material, table S4). SIBER analyses of shallow (5 m) corals indicated that $\delta^{15}\text{N}$ was a stronger determinant of trophic niche overlap than $\delta^{13}\text{C}$, which can have high overlap despite distinct host and symbiont ellipse areas [6]. SIBER could not be used to assess the effect of depth or host/symbiont genotype on isotopic data due to insufficient sample size (electronic supplementary material, figure S2). To compare the trophic profile of each genotypic division in the *Leptoseris* community across its depth range and to contextualize changes in metabolism [16,24], we characterized the independent relationships of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values across depth.

(b) Autotrophy remains integral to colony survival across mesophotic depths

Host and symbiont $\delta^{13}\text{C}$ decreased with increasing depth for the *Leptoseris* community (figure 2a,b; electronic

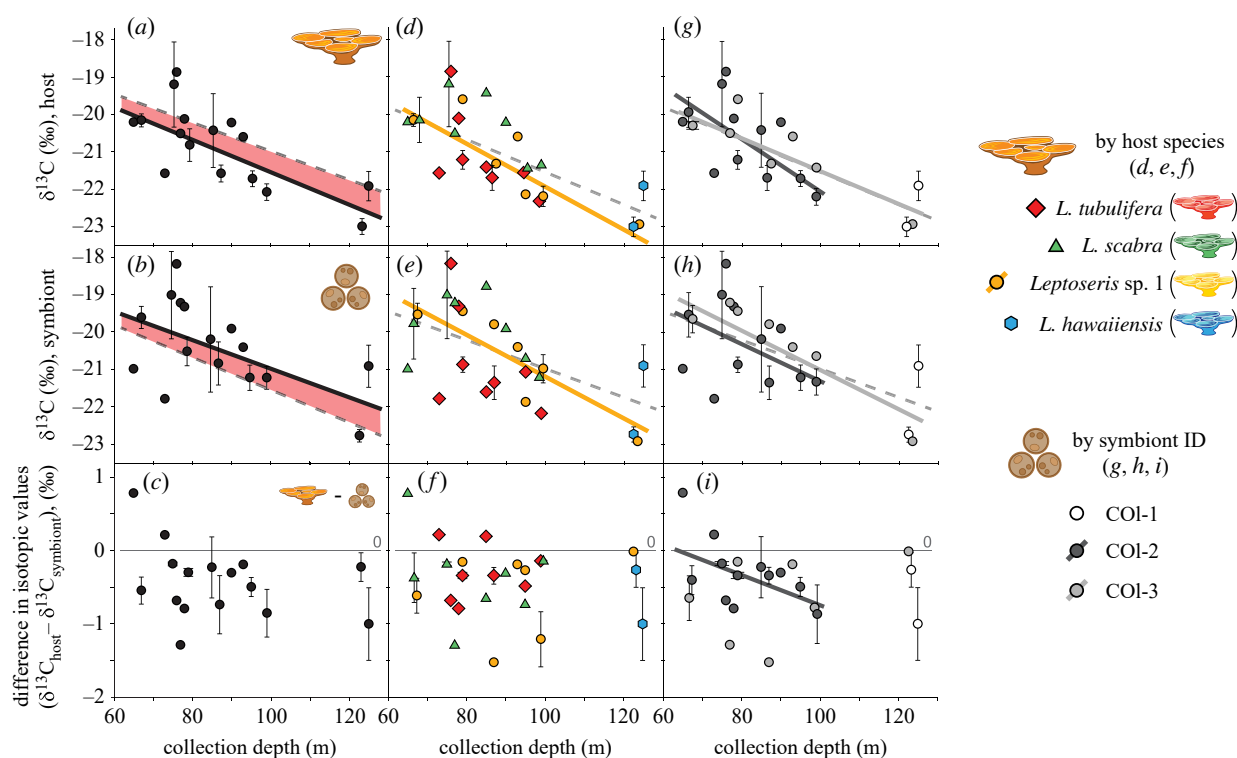


Figure 2. Carbon exchange between hosts and symbionts remains constant for most *Leptoseris* genotypes across depth, supporting sustained reliance on autotrophy. $\delta^{13}\text{C}$ (‰) of (a) host, (b) symbionts and (c) differences between host and symbiont isotopic values for the *Leptoseris* community across depth (m). Points indicate means $\pm$ s.e., binned by collection depth and jittered for clarity. Solid lines show significant linear regressions ($p < 0.05$; electronic supplementary material, table S2). Dashed lines in a and b represent the overlaid regressions for symbionts and hosts, respectively. The red shaded area indicates the difference between host and symbiont $\delta^{13}\text{C}$ values plotted in c. Trend lines in a and b show host $\delta^{13}\text{C}$ was consistently about 0.5‰ lower than symbiont $\delta^{13}\text{C}$ across depths. The same data by (d–f) host species or (g–i) symbiont haplotype show host and symbiont $\delta^{13}\text{C}$ and the difference between them. Re-categorizing points changed some of their values and total counts across plots. Dashed lines in d–e and g–h indicate the community trends shown in a and b, respectively. Solid lines show significant linear regressions for host species (d–f) and algal haplotype (g–i) ($p < 0.05$; electronic supplementary material, table S2). Zero-lines (grey) in c, f and i indicate no isotopic difference between host and symbiont fractions.

supplementary material, table S2), as observed in other mesophotic corals [4,12,14,15]. However, host and symbiont $\delta^{13}\text{C}$ did not diverge [27]; instead, host $\delta^{13}\text{C}$ was consistently approximately 0.5‰ lower than symbiont $\delta^{13}\text{C}$ across depth (figure 2c). Decreases in $\delta^{13}\text{C}$ of suspended POM across depth associated with bacterial remineralization are expected to decrease host $\delta^{13}\text{C}$ via heterotrophy at deeper depths [12,49]. As symbionts primarily obtain carbon from the host (e.g. via metabolites including CO_2), the decrease in symbiont $\delta^{13}\text{C}$ across depth is probably linked to the decrease in host $\delta^{13}\text{C}$ [12,14]. Across depth, heterotrophy provides additional carbon to the host that is not shared with the symbionts, explaining why host $\delta^{13}\text{C}$ remains more negative than symbiont $\delta^{13}\text{C}$ [3,12,27]. However, the consistent relationship between host and symbiont $\delta^{13}\text{C}$ suggests no relative change in photosynthates translocated from symbionts to hosts across depth [12,16,27]. Grouping by host species (figure 2d–f) and symbiont haplotypes (figure 2g–i) displayed a consistent trend of decreasing host and symbiont $\delta^{13}\text{C}$ values with depth (electronic supplementary material, table S2). *Leptoseris hawaiiensis* and COI-1 were only found from 123 to 125 m (figure 2d–f,g–i); omitting *L. hawaiiensis* from our regressions of $\delta^{13}\text{C}$ versus depth across coral hosts and algal haplotypes increased the steepness of host and symbiont slopes by 33% and 34%, respectively (electronic supplementary material, table S3). However, the slopes exhibited across coral hosts and algal haplotypes remained statistically similar to one another without *L. hawaiiensis* (model $F = 16.7$, model $p < 0.0001$, slope interaction $p = 0.71$).

The only exception to the trends in $\delta^{13}\text{C}$ across depth was that the negative $\delta^{13}\text{C}_{\text{host}} - \delta^{13}\text{C}_{\text{symbiont}}$ values decreased with increasing depth in COI-2 colonies (figure 2i; electronic supplementary material, table S2). The increasing difference in $\delta^{13}\text{C}$ between COI-2 fractions across depth suggest a haplotype-specific trophic shift for COI-2 colonies towards increased reliance on heterotrophy [27]. Lower dark-adapted photosynthetic yield in some species harbouring COI-2 symbionts (70–100 m) relative to COI-1 at the deepest depths (110–120 m) may indicate a reduced capacity for autotrophy [3], possibly explaining why COI-2 colonies were not found below 100 m. The negative isotopic shift of approximately 1‰ difference for this haplotype between 65 and 100 m is notably less than the negative shift in $\delta^{13}\text{C}_{\text{host}} - \delta^{13}\text{C}_{\text{symbiont}}$ reported (approx. 2–8‰) for other mesophotic corals [12,14,15,27]. For the rest of the community, the similarity in slope for hosts and symbionts (electronic supplementary material, table S2), despite drastic decreases in available light, suggests that autotrophy remains crucial for *Leptoseris* across depth [12,15]. Differences between host and symbiont $\delta^{13}\text{C}$ for this *Leptoseris* community (figure 2c,f,i; mean $(\delta^{13}\text{C}_{\text{host}} - \delta^{13}\text{C}_{\text{symbiont}}) \pm \text{s.e.} = -0.5 \pm 0.1\text{‰}$ across depths) are similar to those reported for some shallow reef corals at just 5 m (mean $(\delta^{13}\text{C}_{\text{host}} - \delta^{13}\text{C}_{\text{symbiont}})$ approximately 0.4–0.6‰ for three species, [6]). Differences in trophic strategy among those shallow-reef species were determined by differences in $\delta^{15}\text{N}$ between host and symbiont fractions [6]. Therefore, nitrogen must be considered with $\delta^{13}\text{C}$ to substantiate and contextualize relative trophic rates for mesophotic *Leptoseris*.

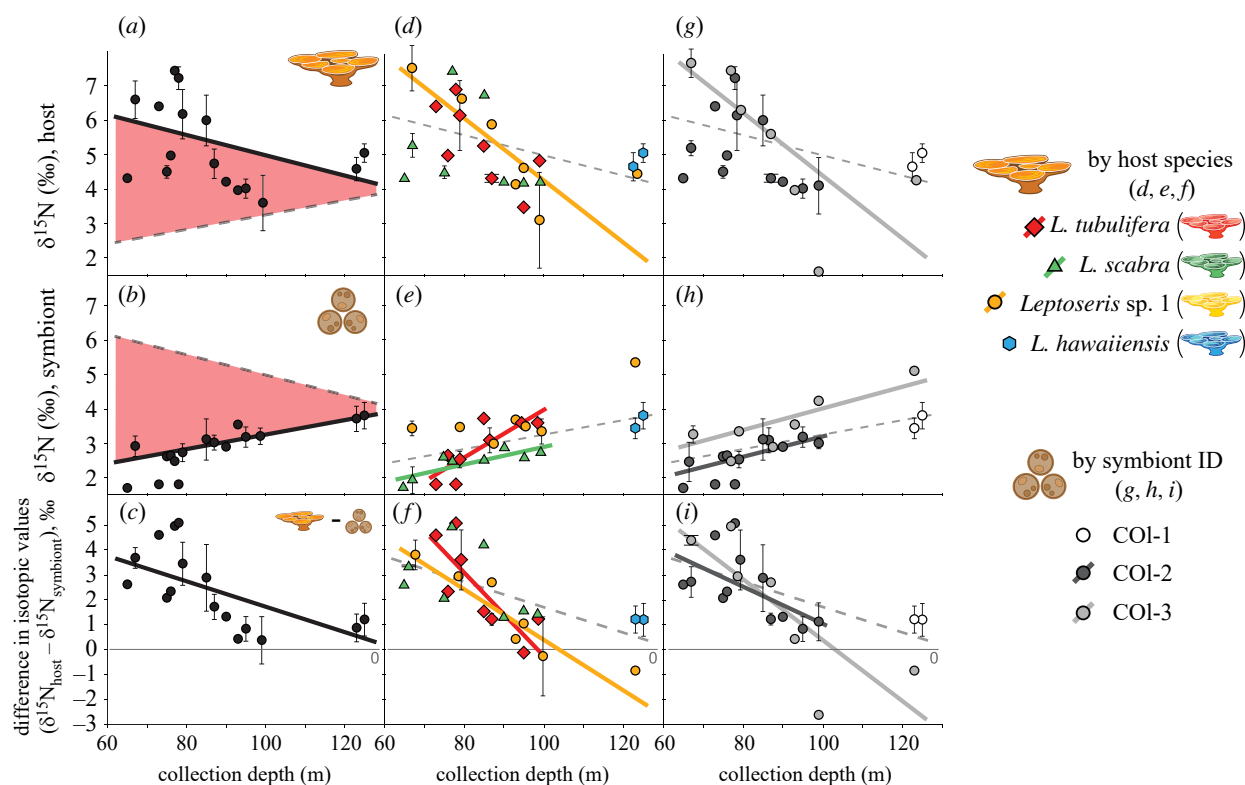


Figure 3. Host $\delta^{15}\text{N}$ and symbiont $\delta^{15}\text{N}$ converge across depth, providing further evidence for sustained autotrophic reliance through the mesophotic zone. $\delta^{15}\text{N}$ (‰) of (a) host, (b) symbionts and (c) differences between host and symbiont isotopic values for the *Leptoseris* community across depth (m). Points indicate means $\pm$ s.e., binned by collection depth and jittered for clarity. Solid lines show significant linear regressions ($p < 0.05$; electronic supplementary material, table S2). Dashed lines in a and b represent the overlaid regressions for symbionts and hosts, respectively. The red shaded area indicates the difference between host and symbiont $\delta^{15}\text{N}$ values plotted in c. Trend lines in a and b show that host $\delta^{15}\text{N}$ decreased and symbiont $\delta^{15}\text{N}$ increased across depths, converging at approximately 4‰ at 125 m. The same data by (d–f) host species or (g–i) symbiont haplotype show host and symbiont $\delta^{15}\text{N}$ and the difference between them. Re-categorizing points changed some of their values and total counts across plots. Dashed lines in d,e and g,h indicate the community trends shown in a and b, respectively. Solid lines show significant linear regressions for host species (d–f) and algal haplotype (g–i) ($p < 0.05$; electronic supplementary material, table S2). Zero-lines (grey) in c, f, and i indicate no isotopic difference between host and symbiont fractions.

$\delta^{15}\text{N}$ values across depth suggest nitrogenous resources are shared between *Leptoseris* hosts and their algal symbionts through the mesophotic zone. Unlike previous studies, where host and symbiont $\delta^{15}\text{N}$ diverged [3] or remained constant [4,15] with increasing depth, we found that host and symbiont $\delta^{15}\text{N}$ converged through the mesophotic zone (figure 3a,b; electronic supplementary material, table S2). For the *Leptoseris* community, host $\delta^{15}\text{N}$ decreased with depth (figure 3a), while symbiont $\delta^{15}\text{N}$ increased with depth (figure 3b). Consequently, the difference between host and symbiont $\delta^{15}\text{N}$ decreased with increasing depth, approaching zero (figure 3c) and causing host and symbiont $\delta^{15}\text{N}$ to converge at an average of approximately 4‰ for both fractions at 125 m (figure 3a,b). Like $\delta^{13}\text{C}$, host and symbiont $\delta^{15}\text{N}$ trends across depth were well-conserved across species and haplotypes. While not all colony host species/algal haplotypes produced significant regressions for $\delta^{15}\text{N}$ across depth, all significant regressions matched the community trends, and all non-significant relationships conformed to the overall pattern (figure 3d–i; electronic supplementary material, table S2). Omitting *L. hawaiiensis* did not change the statistical significance of the trends for $\delta^{15}\text{N}$ regressions across coral hosts and algal haplotypes across depth, and host and symbiont $\delta^{15}\text{N}$ regressions containing all other species/haplotypes combined maintained negative and positive slopes across depth, respectively (electronic supplementary material, table S3).

Our $\delta^{15}\text{N}$ results contradict any expected shift to heterotrophic dependence at depth. SIBER analyses suggested that heterotrophy is the primary nutritional source for our

Leptoseris community; however, if *Leptoseris* colonies relied more on heterotrophy relative to autotrophy with increasing depth, $\delta^{15}\text{N}$ values of hosts and symbionts should become more distinct. Nitrogenous resources acquired heterotrophically by the host are expected to decouple host and symbiont $\delta^{15}\text{N}$ values as autotrophic contributions from the symbionts to the host decrease [6,18,19]. The $\delta^{15}\text{N}$ of both suspended and sinking POM are expected to increase across depth as a function of processes including ^{14}N -preferred bacterial degradation [49,50]. This degradation probably contributed to $\delta^{15}\text{N}$ increases of 1‰ in coral skeletons observed from 5 to 105 m in Palau, mirroring increases in the $\delta^{15}\text{N}$ of suspended POM [49]. Around Maui, increases in mean macroalgal $\delta^{15}\text{N}$ from shallow (0–30 m; 2.4‰) to mesophotic depths (30–117 m; 2.9‰) were attributed to submarine groundwater discharge potentially linked to anthropogenic nutrient pollution [30]. All such environmental increases in source $\delta^{15}\text{N}$ and hypothesized increases in heterotrophy through the mesophotic zone [27] should increase host $\delta^{15}\text{N}$ alone or increase both host and symbiont $\delta^{15}\text{N}$ across depth. Yet, in our *Leptoseris* community, host $\delta^{15}\text{N}$ did not increase for any genotype across mesophotic depths (figure 3a,d,g). Along with $\delta^{13}\text{C}$, these $\delta^{15}\text{N}$ findings suggest that relative rates of heterotrophy are not increasing or replacing autotrophy as depth increases. If heterotrophy decreased relative to autotrophy, $\delta^{13}\text{C}$ values between fractions should have become more similar across depth. Instead, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ regressions reveal a consistent reliance on autotrophy across depth.

These $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ trends across depth differed from Padilla-Gamiño *et al.* [3], which found that *Leptoseris* host and symbiont regressions for both stable isotopes diverged with increasing depth, supporting increased heterotrophy at depth. In that study, *Leptoseris* stable isotope samples were collected earlier, in January–April 2009–2010, and may have included any of the six *Leptoseris* species from the mesophotic zone of the ‘Au‘au Channel, including *L. yabei* and *L. papyracea*. Both were absent from our samples collected in February–March 2011. Seasonal variability in the environment at collection time may add to isotopic differences between studies. Over half (approx. 54%) of isotopic samples from the prior study [3] were collected in April when subsurface current speed can increase in Hawaiian mesophotic habitats approximately 50 m deep [38]. This current can increase mixing of zooplankton and detritus across mesophotic depths [27,51], potentially increasing heterotrophy relative to our winter collections. Differences among submersible collection sites and microhabitats in the ‘Au‘au Channel between years may have compounded trophic differences between these two distinct collections due to spatial variations in submarine groundwater discharge [30] and thermal variability [16].

Our results are supported by studies of Caribbean *Montastraea cavernosa* corals across a shallow-to-mesophotic gradient. Mesophotic *M. cavernosa* grow flat, plating morphologies [15,31] like *Leptoseris* optimizing incident irradiance at depth [31]. Decreased host $\delta^{13}\text{C}$ across depth (3–91 m) was originally attributed to increased heterotrophy and reliance on zooplankton [15,27]. Subsequent analyses found that relative compositions of heterotrophic sterol markers and phytosterols from symbionts remained the same between 18–20 m and 55–60 m, indicating primary reliance on autotrophy to at least 60 m [31]. Similarly, while host and symbiont $\delta^{13}\text{C}$ decreased with depth in our *Leptoseris* community, relative differences between host and symbiont $\delta^{13}\text{C}$ did not change from 65 to 125 m (figure 2c).

Both skeletal microstructures that amplify scattered light and low polyp density that lessens self-shading support photoacclimatization of *Leptoseris* at extreme mesophotic depths [5,21–24]. These adaptations may benefit mesophotic corals relative to other coral morphotypes. While mounding corals maximize theoretical gross primary productivity across depth, integrated light scattering demonstrates that plating species have higher photosynthetic potential across bathymetric slopes into the mesophotic zone than mounding or branching species [52]. Plating could optimize symbiont photosystems within coral tissues at depth [53] and maximize carbon translocation under low irradiance [17], enabling autotrophic advantages over other morphotypes in these systems. Our results support that autotrophy persists in these corals across depth, possibly facilitated by plating and other morphological adaptations.

(c) Nitrogen exchange could sustain autotrophic capacity at extreme mesophotic depths

The convergence between *Leptoseris* host and symbiont $\delta^{15}\text{N}$ values with increasing depth suggests that nitrogenous resources are increasingly shared between partners at depth (figure 4a–d), similar to *Stylophora pistillata* [4,19] and *Favia fava* [4]. In coral symbiosis, hosts typically have a higher $\delta^{15}\text{N}$ relative to their symbionts as ^{14}N -ammonium waste is preferentially excreted or shared with symbionts (figure 4a)

[19,54]. At depths less than 90 m in the ‘Au‘au Channel, current speeds of at least $10\text{--}15\text{ cm s}^{-1}$ occur during the winter and may have coincided with our collections, exposing *Leptoseris* to abundant heterotrophic nitrogen (e.g. zooplankton/detritus) [27,38,51]. Mean $\delta^{15}\text{N} \pm \text{s.e.}$ of *Leptoseris* host and symbiont fractions across depth ($4.5 \pm 0.4\text{‰}$ at 65 m, decreasing to $3.4 \pm 0.4\text{‰}$ at 100 m; electronic supplementary material, table S4) resemble the upper bound of 3–3.5‰ for sinking POM at 100–150 m depth off the Hawaiian Islands [50]. *Leptoseris* may also feed on suspended particulate nitrogen (PN) [49]; however, $\delta^{15}\text{N}_{\text{PN}}$ measured at the nearby ALOHA station was much lower than host $\delta^{15}\text{N}$ values (mean $\delta^{15}\text{N}_{\text{PN}} = -0.3\text{--}2.2\text{‰}$ from 43 to 175 m, respectively; [50]). Mean host $\delta^{15}\text{N}$ at 65 m (approx. 6.3‰) is in the trophic range of 2.5–3.5‰ higher than sinking POM expected if these corals acquire nitrogen heterotrophically [6,50,55,56]. Therefore, nitrogen may be obtained at shallower depths by coral hosts through heterotrophic feeding while carbon is still obtained through photosynthesis, reflected in consistent $\delta^{13}\text{C}_{\text{host}}\text{--}\delta^{13}\text{C}_{\text{symbiont}}$ across depth (figure 2). High heterotrophy on POM supplying abundant nitrogen at shallower depths could occur with relatively high rates of autotrophy requiring relatively low nitrogen exchange and producing distinct host and symbiont $\delta^{15}\text{N}$ values.

By contrast, increased exchange of limited nitrogen could explain the similarity in host and symbiont $\delta^{15}\text{N}$ values in the lower mesophotic zone (figure 4b). At depths greater than 90 m, ‘Au‘au Channel currents decrease to near-stagnant levels throughout the year [38], potentially limiting nitrogenous resources including POM [27,51]. Nitrogen limitation in both hosts and symbionts has been observed in mesophotic *Stylophora pistillata* (50 m) compared with shallower conspecifics (5 m) [8]. Increased flow of nitrogen exchanged from symbionts to the host should decrease host $\delta^{15}\text{N}$ [18]. Therefore, decreased excretion of nitrogen from the holobiont and increased homogenization between symbionts and hosts could explain the convergence of host and symbiont $\delta^{15}\text{N}$ values at depth (figure 3). Previous experimentation indicated that symbionts of Red Sea *Leptoseris* spp. corals (82–90 m) transferred at least 50% of N acquired from dissolved inorganic nitrogen (DIN) to their hosts after an 8-hour incubation period [7]. The capacity for Hawaiian *Leptoseris* to increasingly exchange nitrogen [7,31] between host and symbiont compartments may enable autotrophy at deeper depths despite reduced light availability. Lower rates of photosynthetic electron transport at the deepest collection depths [3] probably decrease photosynthate production compared with shallower colonies, as observed for Red Sea *Leptoseris* spp. [7]. Nitrogen limitation linked to reduced heterotrophy, matched with a light-limited decrease in photosynthate production, could explain how relative carbon exchange between hosts and symbionts seems to remain consistent while nitrogen exchange increases across depth (figures 2–4).

(d) Implications for mesophotic Hawaiian reefs

Reliance on photosynthesis at depth may contribute fundamentally to the skeletogenesis of mesophotic reefs. Mesophotic colonies of *Leptoseris fragilis* from the Red Sea have maximum growth rates of 0.8 mm yr^{-1} , growing up to 8–10 cm in diameter [21]. *Leptoseris* colonies in Hawai‘i can grow diameters greater than 1 m with growth rates of $1\text{--}2.5\text{ cm yr}^{-1}$, comparable to some shallow water corals

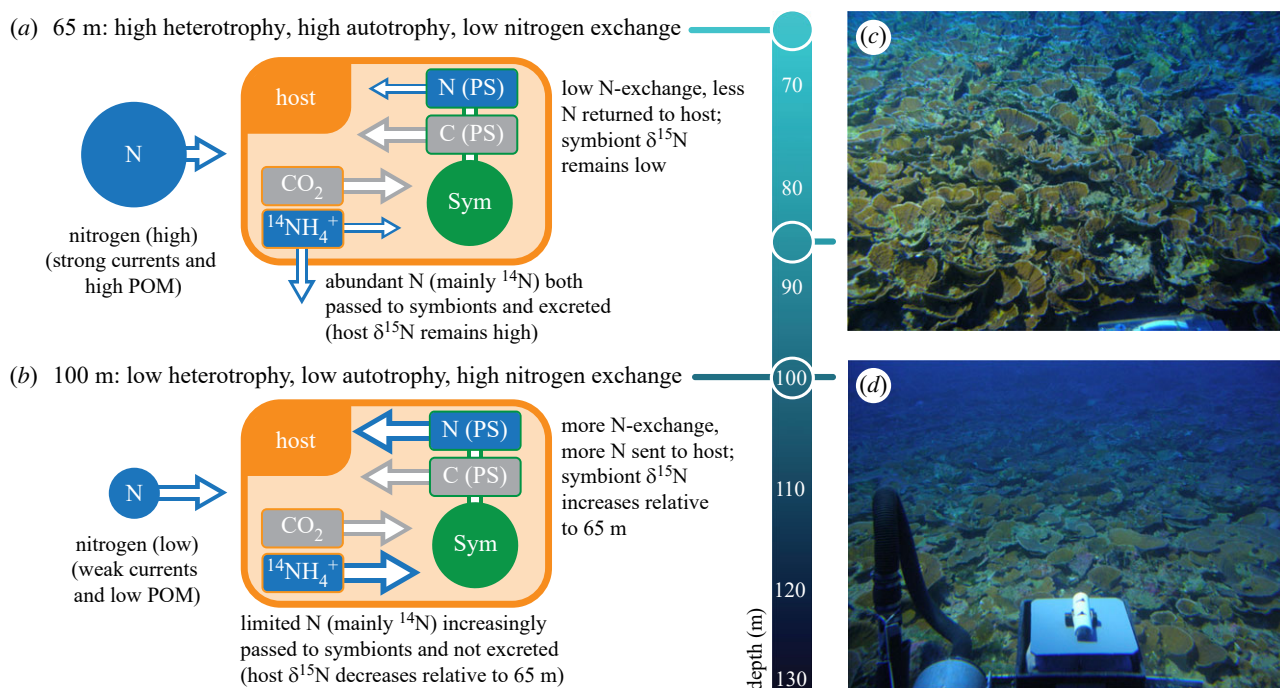


Figure 4. Together, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ explain how nitrogen exchange supports autotrophy at depth. Schematic hypothesis for our results across collection depth. *Leptoseris* hosts can obtain carbon and nitrogen heterotrophically, most likely from POM. Symbionts can supply carbon and nitrogen to hosts autotrophically by taking dissolved inorganic carbon and nitrogen from the water column and translocating photosynthates (PS) to the host. (a) At 65 m, with abundant nitrogen (N), NH_4^+ waste can be excreted from the coral and/or symbionts. Symbionts do not readily share/exchange N with the host due to high abundance of N. Preferential excretion of $^{14}\text{NH}_4^+$ and limited N sharing increase host $\delta^{15}\text{N}$; as ^{14}N is excreted, host $^{15}\text{N} : ^{14}\text{N}$ increases. Symbiont $\delta^{15}\text{N}$ remains low as symbionts transfer less N to hosts, producing lower symbiont $^{15}\text{N} : ^{14}\text{N}$. Heterotrophy contributes more to *Leptoseris* energy inputs than autotrophy (figure 1). However, relative carbon exchange between hosts and symbionts is consistent across depth (i.e. symbiont $\delta^{13}\text{C}$ approximately 0.5‰ higher than host $\delta^{13}\text{C}$ across depth; figure 2). Therefore, high heterotrophy on POM that supplies high nitrogen inputs at 65 m corresponds with proportionally high rates of autotrophy. (b) At 100 m, weak currents limit POM, so *Leptoseris* increase N exchange between host and symbionts. Excretion is limited and hosts share higher amounts of $^{14}\text{NH}_4^+$ waste to the symbionts (larger $^{14}\text{NH}_4^+$ arrow towards Sym). Symbionts transfer more N to hosts. N exchange homogenizes host and symbiont $^{15}\text{N} : ^{14}\text{N}$ ratios, decreasing host $\delta^{15}\text{N}$ and increasing symbiont $\delta^{15}\text{N}$ compared with 65 m (figures 3 and 4a). Since symbiont $\delta^{13}\text{C}$ remains approximately 0.5‰ higher than host $\delta^{13}\text{C}$ (figure 2), reduced heterotrophy at 100 m coincides with a proportional reduction in autotrophy, consistent with light limitation at depth. *Leptoseris* reefs from the 'Au'au Channel, Hawai'i at (c) 86 m and (d) 100 m (submersible photographs courtesy of the Hawai'i Undersea Research Laboratory).

[3,38,57]. Hawaiian *Leptoseris* colonies probably invest heavily in skeletal extension to increase lateral growth relative to thickness due to the stable environment of their deep habitat that is minimally impacted by wave action [38,45]. Low metabolic demand for carbon resources [16,28], coupled with increased nitrogen exchange, may enable *Leptoseris* to survive and grow in light-limited conditions. High skeletal growth rates observed in Hawaiian *Leptoseris* communities could be maintained by conserving photosynthates required for light-enhanced calcification [21,22,58,59]. Investment in skeletal microstructures that enhance scattering and amplify light for symbionts [5,22] could enable skeletal growth to synergistically fuel photosynthesis in a positive feedback loop at mesophotic depths.

Based on our stable isotope analyses, trophic strategy cannot explain any competitive advantage of one *Leptoseris* species relative to others at least to 125 m in Hawaiian mesophotic reefs. We did not find that depth partitioning of *Leptoseris* corals in the 'Au'au Channel corresponded to niche partitioning of trophic strategies. While heterotrophy may serve as the main trophic input of carbon and nitrogen for *Leptoseris* across depths, the ability to photosynthesize under low-light conditions may enable coral survival and growth throughout the mesophotic zone. Although *Leptoseris* species in this community share host macromorphologies [5,23,24], niche diversity of their microbial communities, including dark-adapted yields of algal symbionts [3,35] and nitrogen

fixation and exchange by bacteria [60], may drive the depth partitioning of different *Leptoseris* genotypes [3]. Characterizing microbial diversity and function in corals across mesophotic depths could help explain the sustained autotrophy extending across host species and algal haplotypes.

This study provides a novel example of isotopic data over a wide mesophotic gradient [16,27,28] where examining carbon and nitrogen, both independently and combined, contributed to the understanding of trophic strategies that sustain coral survival in light-limited environments. Conservation strategies prioritizing water clarity by minimizing shoreline run-off could bolster the autotrophic capacity of *Leptoseris* colonies. An enhanced perspective of mesophotic reefs as both exponentially light-limited and persistently light-dependent coral communities could improve our ability to understand and conserve the rich biodiversity and vast extent of these largely unexplored ecosystems.

Ethics. Coral samples were collected under SAP permit 2009-72 from the Department of Land and Natural Resources, State of Hawai'i. However, the majority of corals were collected in US Federal Waters and did not require a permit for collection.

Data accessibility. Copies of all data files have been submitted to Dryad: <https://doi.org/10.5061/dryad.f1vhhmh0z> [61].

Supplementary material is available online [62].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. C.H.B.: conceptualization, formal analysis, investigation, visualization, writing—original draft, writing—review and editing; J.L.P.: conceptualization, funding acquisition, project administration, supervision, writing—review and editing; H.L.S.: funding acquisition, project administration, writing—review and editing; M.S.R.: writing—review and editing; C.M.S.: funding acquisition, writing—review and editing; R.D.G.: funding acquisition; L.J.R.: conceptualization, funding acquisition, investigation, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. The authors declare no competing interests.

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