Partner switching and metabolic flux in a model cnidarian–dinoflagellate symbiosis

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Metabolite exchange is fundamental to the viability of the cnidarian–Symbiodiniaceae symbiosis and survival of coral reefs. Coral holobiont tolerance to environmental change might be achieved through changes in Symbiodiniaceae species composition, but differences in the metabolites supplied by different Symbiodiniaceae species could influence holobiont fitness. Using 13C stable-isotope labelling coupled to gas chromatography–mass spectrometry, we characterized newly fixed carbon fate in the model cnidarian Exaiptasia pallida (Aiptasia) when experimentally colonized with either native Breviolum minutum or non-native Durusdinium trenchii. Relative to anemones containing B. minutum, D. trenchii-colonized hosts exhibited a 4.5-fold reduction in 13C-labelled glucose and reduced abundance and diversity of 13C-labelled carbohydrates and lipogenesis precursors, indicating symbiont species-specific modifications to carbohydrate availability and lipid storage. Mapping carbon fate also revealed significant alterations to host molecular signalling pathways. In particular, D. trenchii-colonized hosts exhibited a 40-fold reduction in 13C-labelled scyllo-inositol, a potential interpartner signalling molecule in symbiosis specificity. 13C-labelling also highlighted differential antioxidant- and ammonium-producing pathway activities, suggesting physiological responses to different symbiont species. Such differences in symbiont metabolite contribution and host utilization may limit the proliferation of stress-driven symbioses; this contributes valuable information towards future scenarios that select in favour of less-competent symbionts in response to environmental change.

1. Background

A symbiosis based on the mutualistic exchange of metabolites between reef-building corals and dinoflagellate endosymbionts of the family Symbiodiniaceae [1] is fundamental to the ecological success of coral reefs in otherwise unproductive tropical oceans [2]. The coral host supplies dissolved inorganic carbon (Ci), nitrogen and other compounds to support symbiont photosynthesis, growth and nitrogen assimilation (reviewed in [3]). In return, the Symbiodiniaceae cells translocate a portion of their photosynthate to the coral host, contributing to host respiration, growth, calcification and reproduction [4–6]. To date, the symbiont-derived metabolites identified in cnidarian tissues range from soluble, low-molecular-weight compounds such as glucose and amino acids [4,6,7], to more complex molecules such as fatty acids, organic acids and lipids [5,8–10].

The highly diverse family Symbiodiniaceae [1] exhibits physiological variation within and between genera, including in the flow of organic carbon (C,) to the host and thermal tolerance [11–13]. This diversity has led to the suggestion that symbiont identity may play an important role in determining the adaptive capacity of corals in response to ocean warming [11,14]. Changes in the symbiont community are commonly restricted to those Symbiodiniaceae taxa already present within the coral host (symbiont ‘shuffling’), with limited evidence for the establishment...
and persistence of novel symbioses with algal symbionts acquired from the surrounding seawater (symbiont ‘switching’) [15]. Molecular and physiological evidence suggests that for symbiont switching to occur, potential novel symbionts must successfully navigate a gauntlet of host immune responses [16–18]. Once candidate symbionts are enveloped within the host’s cells by the symbiosome membrane complex, the persistence of the symbiosis requires sufficient nutritional exchange across this membrane complex to provide the host with an energetic advantage [18]. Long-term maintenance of a functional symbiosis probably requires physiological advantages and ecological benefits to the entire holobiont consortium of the coral, symbiotic dinoflagellates and other symbiotic microbes [11,19].

Recent molecular and physiological evidence revealed that switching to a heterologous (i.e. non-native) symbiont comes at a physiological cost to the cnidarian host, including elevated catabolism of fixed C stores, significant reduction of calcification rates and immune responses to the unfamiliar symbiont [18,20,21]. Whether such metabolic consequences are a result of differences in the identity and/or abundance of metabolites supplied by the symbiont remains unclear. In this regard, the amount of C, translocated to the host has been shown to depend on the resident Symbiodiniaceae species [13,22,23]. For example, conspecific corals may acquire less photosynthate from symbionts from the genus Durusdinium than from symbionts from other genera, leading to slower coral growth [22,24,25]. Furthermore, Symbiodiniaceae metabolite profiles vary in composition and quantity by genotype when in culture [26], raising the possibility that there may be differences in the identity and abundance of compounds translocated to the cnidarian host when in hospite.

To test whether the abundance and/or diversity of fixed carbon compounds released to the host varies with symbiont species, we used the sea anemone Exaiptasia pallida (commonly referred to as ‘Aiptasia’), an established model system for the study of cellular interactions in the cnidarian–dinoflagellate symbiosis [27]. We compared the relative abundance and identity of 13C-labelled photosynthetically fixed carbon in Aiptasia when experimentally colonized by the homologous (i.e. native) Brevediurn minutum (ITS2 type B1) or the heterologous Durusdinium trenchii (ITS2 type D1a). Durusdinium trenchii has been observed to repopulate corals following a bleaching event and to confer thermal resilience on the host [11,28]; however, there is doubt about the long-term potential for this relatively unproductive symbiont to contribute to overall coral reef health and growth [18,21,25,29]. Durusdinium trenchii is therefore an especially interesting candidate for investigations of the nutritional implications of partner switching [11,28]. This comparative approach reveals key differences in the identity and utilization of metabolic products provided by the symbionts, which is likely to impact host energy storage and catabolism, and molecular signalling pathways. Given that metabolic exchange is a central feature of the symbiosis, such differences may impact the fitness of novel host–symbiont combinations, and thus the potential for symbiont switching to provide an adaptive mechanism by which reef corals can respond to environmental change.

2. Material and methods

(a) Experimental organisms

Aiptasia (n ~ 750), containing the homologous symbiont B. minutum (ITS2 type B1), were collected from a clonal laboratory stock, strain NZ1 [18]. Anemones were maintained in 0.22 μm aerated filtered seawater (FSW) at 25°C, with light provided by fluorescent lamps at approximately 95 μmol quanta m–2 s–1 on a 12 L: 12 D cycle. Anemones were fed twice a week with freshly hatched Artemia sp. nauplii. Anemones were rendered aposymbiotic using the methylthion treatment method [30]. Aposymbiotic status was confirmed by confocal microscopy (Olympus Provis AX70, at 100× magnification). Two different Symbiodiniaceae cultures were used: the homologous species B. minutum (ITS2 type B1, culture ID FLAp2) and a heterologous species D. trenchii (ITS2 type D1a; culture ID Ap2). FLAp2 was originally isolated from E. pallida and has been shown to successfully colonize NZ1 anemones [13,18].

Symbiodiniaceae cultures were grown in silica-free f/2 medium at 25°C and an irradiance of 100 μmol quanta m–2 s–1 on a 12 L: 12 D cycle for six weeks before use in the colonization study.

(b) Colonization of Aiptasia by Symbiodiniaceae

Anemones were starved for 7 days prior to inoculation with Symbiodiniaceae. Approximately 240 aposymbiotic Aiptasia per treatment were inoculated during the midpoint of the light cycle with either B. minutum or D. trenchii by applying Symbiodiniaceae cultures at a similar cell density (approx. 3 × 106 cells ml–1) combined with a dilute suspension of Artemia nauplii to induce feeding. The inoculation procedure was repeated weekly for three more weeks (i.e. four weeks total), with temperature and light regimes maintained as per pre-colonization; however, the anemones were only fed during the symbiont colonization procedure to encourage symbiont uptake.

(c) Stable-isotope (13C) incubations

Stable-isotope incubations were based on the methods of Hillyer et al. [6]. Anemones were starved for one week prior to incubation with the stable-isotope tracer (i.e. five weeks after the initial Symbiodiniaceae inoculation). Before the start of the light cycle, the FSW was replaced with 250 ml of FSW containing either 4 mM 99% 13C-labelled sodium bicarbonate or 4 mM 12C sodium bicarbonate (Sigma–Aldrich, New Zealand). Seawater pH was adjusted to that of the original FSW (pH 8.2). Each incubation (12°C/13C) and symbiote (aposymbiotic/B. minutum/D. trenchii) comprised six replicates (total n = 36).

Samples were incubated for 5 h under the same irradiance (approx. 95 μmol quanta m–2 s–1) and temperature (25°C), to allow for sufficient photosynthetic activity and incorporation of a high enough level of the carbon label for robust detection of metabolites, without significant enrichment of host lipids [6]. At the end of the incubation, the FSW was gently discarded, and the beakers sealed and flash frozen in liquid nitrogen to quench metabolism (approx. 10 s). Samples were stored at –80°C until processing.

(d) Sample processing and intracellular metabolite extraction

Samples were processed and metabolites were extracted as described by Matthews et al. [18]. Each sample was drawn from a pool of 20 individual anemones to ensure sufficient biomass for metabolite identification and label detection [6]. Each sample of 20 pooled anemones was thawed and mechanically homogenized, and a single aliquot removed for Symbiodiniaceae cell density estimation. Symbionts were pelleted by centrifugation and the cleaned host fractions were frozen at –80°C for 1 h and lyophilized at –105°C for 18 h. DNA buffer (0.4 M NaCl, 0.05 M ethylenediaminetetraacetic acid) with 1% (w/v) sodium dodecyl sulfate was added to the symbiont pellet and frozen at –20°C for symbiont identification.

The semi-polar metabolites were extracted from 30 mg of lyophilized host material and using the internal standard
d-sorbitol-6-\(^{13}\)C. The remaining host fraction was frozen at \(-20^\circ\)C for protein quantification, as determined by a modified Bradford colorimetric method [31].

(e) Analysis of symbiont colonization

Symbiont cell densities were analysed as per Matthews et al. [18]. Protein content was quantified using a modified Bradford colorimetric method [31]. Non-normal or heteroscedastic data were log_{10}-transformed prior to analysis with Student’s \(t\)-tests (\(p < 0.05\), SPSS v. 20, IBM Corp.).

Symbiont identity of each biological replicate was verified by direct sequencing of the internal transcribed spacer region 2 of ribosomal DNA (ITS2 rDNA) and chloroplast 23S rRNA (cp23S) [32]. DNA was extracted using the CTAB–chloroform extraction protocol (https://doi.org/10.17504/protocols.io.dzyq7vv). Samples were immediately used for PCR, using a published thermal cycling regimen and reaction mixture, with the primers ITSintfor2 and ITS2rev2, and cp23s1 and cp23s2 [32,33]. PCR products were cleaned with ExoSAP-IT (USB Corporation, OH, USA) and sequenced by Macrogen (Seoul, South Korea). Sequences were aligned with Geneious v. 7.0 (Biomatters, Auckland, New Zealand), and a BLAST search was performed against Symbiodiniaceae ITS2 and cp23s sequences in GenBank to confirm symbiont identity.

(f) Online derivatization and gas chromatography–mass spectrometry analysis

Concentrated samples were derivatized and analysed (electronic supplementary material, methods S1). Gas chromatography–mass spectrometry (GC–MS) was performed using an automated online derivatization method, adapted from established protocols [34]. Compounds were identified using an in-house mass spectral library and retention-time standard mixtures comprised \(n\)-alkanes (electronic supplementary material, methods S2).

(g) Data analysis and validation

Metabolite data were extracted, analysed and normalized (electronic supplementary material, methods S2). Stable-isotope enrichment analysis was conducted following methods adapted from Hillyer et al. [6]. Spectral components in a given sample were separated, detected and identified during deconvolution using Metabolite Detector software [35], with the application of retention indices and alkane standard runs. Identifications were manually confirmed with AMDIS and MassHunter. Unidentified labelled compounds were analysed for pairwise similarity to known compounds using the mass isotopolome analyser (MIA) software [36] (electronic supplementary material, methods S3). The technical replicate data files of each \(^{13}\)C-labelled and non-labelled \(^{13}\)C samples were then loaded into Non-Targeted Tracer Fate Detector (NUTFD) software for \(^{13}\)C-enrichment analysis [37].

The mass isotopomer distribution (MID) (i.e. \(M_0 + M - M + 10\)) for every detected and labelled fragment ion was calculated using the strictest detection settings in NUTFD, in order to minimize the generation of false positives [37,38]. Compounds were considered enriched if at least two spectral fragments were detected under at least one experimental treatment, \(R^2 = 0.98\), minimal enrichment = 5, and maximal fragment deviation = 0.02. Any compounds identified as being labelled in the apsymbiotic state were excluded from further analysis. All host metabolites that were labelled in the symbiotic, but not apsymbiotic, state were therefore assumed to be either a translocated compound or a downstream product of a translocated compound.

For each labelled compound, a characteristic ion was manually selected; this was typically the most abundant or closest to the heaviest ion (the ion with the greatest \(m/z\)). Mean values and the standard error mean for each MID associated with this ion were then calculated for each labelled compound, taking into account the natural \(^{13}\)C isotope abundance (approx. 1.1% of the total ion distribution). The relative pool size of each compound between treatments was calculated for the unlabelled (\(M_0\)) and labelled (\(M_i\)) fractions, using the following equations:

\[
M_0 = \text{Normalized metabolite abundance} \times (M + 0) \\
M_i = \text{Normalized metabolite abundance} \times (1 - M + 0)
\]

For each enriched metabolite, assumptions of normality and homoscedasticity were assessed using Shapiro–Wilk and Levene’s tests, respectively. Non-normal or heteroscedastic data were log_{10}-transformed prior to analysis. Student’s \(t\)-tests were used to compare metabolite relative abundance between the symbiont species at the \(p < 0.05\) probability level using the SPSS statistical software (v. 20, IBM Corp.). Data that could not be adequately transformed were analysed using non-parametric Mann–Whitney \(U\) rank-sum tests. The observed differences in metabolite \(^{13}\)C-enrichment and relative abundance were used to infer the activity of associated host metabolic pathways and model the overall cellular response to harbouring different Symbiodiniaceae species. Visual exploration of the metabolites in the respective metabolic pathways was illustrated using VANTED (v. 2.6.2) [39,40].

3. Results

(a) Colonization success

Examination of anemones in the apsymbiotic control treatment revealed no re-establishment of background symbionts at the end of the experiment. Genotyping confirmed that symbionts at the end of the experiment were the same as those originally used for inoculation. Symbiont cell densities in anemones colonized by \(B.\) minutum (1.12 \(\times\) \(10^7\) \(\pm\) 1.2 \(\times\) \(10^6\) cells mg\(^{-1}\) protein) were approximately 1.5-fold higher (\(t = 2.362\), d.f. = 10, \(p = 0.04\)) than in those anemones colonized by \(D.\) trenchii (7.29 \(\times\) \(10^6\) \(\pm\) 1.2 \(\times\) \(10^6\) cells mg\(^{-1}\) protein).

(b) Enrichment analysis of total host metabolite pools

Ten \(^{13}\)C-labelled metabolites including carbohydrates, sugar alcohols, amino acids and fatty acids (figure 1) were consistently detected in both \(B.\) minutum- and \(D.\) trenchii-colonized host metabolite pools. Aspartic acid was labelled in the apsymbiotic host and thus was removed from further analyses (electronic supplementary material, table S1). The inositol isofrom scyllo-inositol contained the highest percentage of label in the \(B.\) minutum-colonized host pools (33% labelled), followed by glucose, mannitol and palmitoleic acid (C16:1n-7) (23% labelled each) (electronic supplementary material, table S2). In \(D.\) trenchii-colonized host pools, glucose and mannitol showed the highest percentage of label (39% labelled each). The greatest difference in a metabolite between the hosts colonized with the different algae was found for scyllo-inositol, for which the labelled fraction was 40-fold more abundant in \(B.\) minutum- than in \(D.\) trenchii-colonized host pools, and the unlabelled fraction was 11-fold more abundant (electronic supplementary material, table S2). Both the labelled fractions and the total relative abundance of glucose, scyllo-inositol, mannitol and myristic acid (C14:0) were significantly more abundant in \(B.\) minutum- than \(D.\) trenchii-colonized host pools (figure 1; electronic supplementary material, table S2). The labelled fractions of glutamate and palmitoleic acid were also significantly more abundant in \(B.\) minutum- than \(D.\) trenchii-colonized host pools (figure 1; electronic supplementary material, table S2).
By contrast, none of the enriched metabolites detected in both symbiotic states were significantly more abundant in the *D. trenchii*-than *B. minutum*-colonized anemones.

**Symbiont-specific metabolite translocation**

Symbiodiniaceae species-specific $^{13}$C-labelling was observed in host metabolite pools. A total of 18 labelled metabolites were identified solely in the *B. minutum*-colonized host pools, with no label being detected in these metabolites in the *D. trenchii*-colonized host pools (figure 1; electronic supplementary material, table S3). These included the sugars fructose, sucrose, maltose and xylose. The saturated fatty acid palmitic acid (C16:0), unsaturated fatty acids oleic acid (C18:1n-9) and linoleic acid (C18:2n-6), and another isoform of inositol, myo-inositol, were also labelled in only *B. minutum*-colonized host tissues.

Three further unidentified metabolites were enriched in *B. minutum*-colonized host tissues only. As metabolically related compounds have highly similar MIDs, pairwise similarity can be used to associate unidentified compounds with identified ones (see electronic supplementary material, methods S3). The mass spectral information for these metabolites (labelled...
as ‘unknown’ followed by their retention times) and the pairwise similarity of their MIDs to other labelled compounds in the dataset provided putative compound classifications [36]: Unknown_19.53 was most similar to fructose (electronic supplementary material, figure S1A), Unknown_22.54 was most similar to palmitic acid (electronic supplementary material, figure S1B) and Unknown_28.8 was most similar to maltose (electronic supplementary material, figure S1C).

By contrast, three labelled metabolites were detected in the metabolite pools of *D. trenchii*-colonized anemones only: tyramine, glutamine and ornithine (electronic supplementary material, table S3). These metabolites exhibited low levels of enrichment, with less than 7% each (electronic supplementary material, table S3).

### (d) Pathway visualization
Metabolite identification, *a priori* knowledge of metabolic pathways and pairwise similarity of MIDs of the unidentified enriched metabolites [36] enabled us to map corresponding biosynthetic pathway activity for each symbiotic treatment (figure 2). In the *B. minutum*-colonized host, labelling occurred as a consequence of a variety of pathways involved in central metabolism (glycolysis and pentose phosphate pathways), and glycerolipid and fatty acid synthesis. By contrast, labelling was primarily associated with the urea cycle and fatty acid catabolism in the *D. trenchii*-colonized host (figure 2).

### 4. Discussion
The diversity and abundance of $^{13}$C-labelled metabolites were lower in the host tissues of animals populated by the heterologous *D. trenchii* than those colonized by homologous *B. minutum*, and host downstream pathways revealed differences in carbon fate and molecular signalling in response to symbiont identity. These differences are summarized in figure 3.

#### (a) Symbiont colonization
*Breviolum minutum* achieved higher cell densities in *Aiptasia* than did *D. trenchii*. This is consistent with other studies.
Differences are a direct result of symbiont genotype. *D. trenchii*-colonized hosts strongly suggest that these identity of enriched compounds between a fold change greater than 2. Furthermore, differences in the abundance are likely to be a direct result of the *B. minutum* harbouring symbiont species (23% and 39% of total glucose in anemones). Glucose pools were highly labelled in anemones hosting either homologous *B. minutum* or heterologous *D. trenchii* (figure 3), providing a fitness benefit over time [5]. By contrast, the notably smaller glucose pools in *D. trenchii*-colonized host tissues suggest: (i) reduced glucose production *de novo*; (ii) reduced glucose translocation from the symbiont; (iii) an increased rate of glucose metabolism (although the absence of enriched glycolysis intermediates suggests that this is unlikely); or (iv) a combination of the above. The greater difference in the unlabelled glucose pools (10-fold) between anemones colonized by the two different symbiont species than the labelled glucose pools (4.5-fold) is additional evidence of long-term nutritional trade-offs of associating with a less-appropriate symbiont [21]. Given the central role of glucose in energy metabolism, its reduced availability when in symbiosis with *D. trenchii* may require the use of stored energy by the host (e.g. via gluconeogenesis) or increased heterotrophy to support host fitness (figure 3) [23]. These results indicate that, despite increased central metabolism, only homologous symbionts translocated sufficient fixed carbon for the Aiptasia host to accumulate storage products under non-stressful temperature and light conditions.

The host exhibited increased fatty acid and lipid biosynthesis when colonized by the homologous *B. minutum*, in contrast to when colonized by heterologous *D. trenchii* (figures 2 and 3). The saturated fatty acids myristate (C14:0) and stearate (C18:0) were enriched in both associations, in contrast to when colonized by heterologous *D. trenchii* (figures 2 and 3). The saturated fatty acids myristate (C14:0) and stearate (C18:0) were enriched in both associations, though both labelled and unlabelled pools of myristate and stearate were more abundant in the presence of *B. minutum* (figure 1). Enrichment of the products of stearic acid elongation (C18:1n-9 and C18:2n-6) and the glycerolipid sn-2 position (D1a) suggest increased heterotrophy e.g. increased lipogenesis and gluconeogenesis (figure 3). The saturated fatty acids myristate (C14:0) and stearate (C18:0) were enriched in both associations, in contrast to when colonized by heterologous *D. trenchii* (figures 2 and 3). 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(figure 3). The observed enrichment of host fatty acid pools in the presence of B. minutum must have resulted from translocation of these metabolites, their precursors or their lipogenesis products, as opposed to degradation of enriched lipid stores. This hypothesis is supported by evidence from a parallel proteome study in which expression of several Niemann-Pick C2 proteins, which are important in sterol transport, was exclusively detected in Aiptasia colonized by B. minutum, and not in those colonized by D. trenchii (A. E. Sproles & C. A. Oakley 2018, unpublished data). Meanwhile, the absence of labelled lipogenesis intermediates in the D. trenchii-colonized host suggests that enriched translocated metabolites, including enriched fatty acids, were immediately used in central metabolism rather than directed to lipid storage. Symbiont genotype has similarly been shown to affect the abundance of stored lipids in corals [42].

These data support previous observations of energetic costs associated with hosting Durusdinium under normal conditions [21,22,25]. Hosts containing less productive symbionts may compensate for reduced availability of fixed C via increased heterotrophic feeding and digestion [43–45], as was recently shown in Aiptasia when colonized by heterologous Durusdinium symbionts [23]. This compensatory mechanism is similar to that observed during coral bleaching, where coral host metabolism becomes more reliant on heterotrophically derived carbon and mobilization of limited stored energy reserves [10,41,46]. Thus, our data not only support the importance of trophic plasticity, but also suggest that, once host energy stores are exhausted, inadequate nutritional exchange is likely to limit the survival and potential proliferation of a novel symbiosis.

(c) Symbiont species affects the composition of labelled fatty acids

The composition of fatty acid and lipid pools in corals has been linked to coral nutrition and health [47,48], and thus, changes to these pools may have the potential to influence the fate of novel symbioses. The mono-unsaturated fatty acid palmitoleic acid (C16:1n-7) is necessary for the synthesis of C16 polyunsaturated fatty acids in corals, and palmitic acid (C16:0) and (C16:1n-7) is necessary for the synthesis of C16 polyunsaturated fatty acids in corals, and palmitic acid (C16:0) and palmitoleic acid represent two of the main components of total lipids in both the cnidarian and dinoflagellate [49–52]. Here, palmitoleic acid was enriched in both B. minutum- and D. trenchii-colonized hosts, although greater enrichment was detected in the former. However, the precursor palmitic acid was only detected as enriched when the host was colonized by B. minutum. Therefore, the enrichment of palmitoleic acid in both symbiotic associations, combined with the absence of enrichment of the precursor palmitic acid in the D. trenchii-colonized hosts, suggests that palmitoleic acid is a form in which saturated fatty acids are translocated from symbiont to host, independent of the symbiotic species tested here. However, targeted analysis of palmitoleic acid synthesis and translocation pathways is required to support these findings.

Linoleic acid (C18:2n-6) was only enriched in B. minutum-colonized host pools. Cnidarians do not possess the Δ12 desaturase enzymes required to synthesize this metabolite from oleic acid (C18:1n-9) [53], while the Breveliam genome does contain an omega-6 fatty acid desaturase enzyme (FAD6E; SmicGene28105, Uniprot ID: P46313 [54]). It is therefore reasonable to assume that linoleic acid is translocated by the homologous symbiont, highlighting a difference between the physiologies of B. minutum and D. trenchii. While the significance of linoleic acid translocation is unclear, several studies have indicated that fatty acid translocation could play a pivotal role in endosymbiosis regulation [8,55]. For example, the formation of host lipid bodies (which have roles in lipid storage and intracellular trafficking) is endosymbiosis dependent [56], and in coral gastrodermal tissue, linoleic acid from the symbiont contributes to the formation of lipid bodies [55]. This supports recent evidence that the contribution of photosynthetically fixed carbon to host lipid bodies is symbiont species-dependent [27]. Linoleic acid is also a precursor molecule to essential fatty acids, including eicosapentaeanoic acid (C20:5n-3), docosaheaxenoic acid (C22:6n-3) and arachidonic acid (C20:4n-6), which are the precursors of a wide variety of short-lived regulatory molecules known as eicosanoids [57,58]. Eicosanoids are hypothesized to participate in general stress response pathways in symbiotic cnidarians [59], and recent evidence indicates that they may feature in host stress responses to novel symbiont colonization [18]. The absence of linoleic acid translocation by D. trenchii may limit eicosanoid stress response signalling in response to the heterologous symbiont, making this an interesting biomarker for future studies.

(d) Symbiont identity affects molecular pathways beyond central energy metabolism

(i) Interpartner signalling

The sugar-alcohol scyllo-inositol was labelled in host pools in both homologous and heterologous associations, but the labelled and unlabelled pools were 40-fold and 10-fold more abundant, respectively, when colonized with B. minutum (electronic supplementary material, table S2). Although the exact role of inositol signalling in the cnidarian–dinoflagellate symbiosis is unclear, some evidence suggests that inositol compounds participate in symbiosis signalling, recognition and dysfunction [6,60–62]. Inositol signalling and metabolism have been described as common pathways in Symbiodiniaceae [61], while different Symbiodiniaceae cultures exhibit different expression patterns of inositol isoforms in response to temperature and light [26]. Moreover, multiple putative sugar/inositol co-transporters have been identified in cnidarian transcriptomes and were upregulated in symbiotic versus apsymbiotic anemones [63–65]. Collectively, these findings highlight an uncharacterized role for inositol compounds in cnidarian–dinoflagellate metabolism and symbiosis signalling. Targeted bioassays, in which the production and translocation of inositol compounds are inhibited, could be used to explore the function, if any, of inositol in the symbiosis.

(ii) Urea cycle activity

Glutamate and arginine enrichment were detected in both symbiotic associations; however, the D. trenchii-colonized anemones also exhibited enrichment in ornithine and glutamine pools, while B. minutum-colonized anemones did not. Glutamate and glutamine can feed into the urea cycle, while ornithine and arginine are components of the cycle (electronic supplementary material, figure S2). While we are unable to distinguish whether labelled compounds were translocated directly from the symbiont or are products of downstream metabolism, the labelling of these four
compounds in *D. trenchii*-colonized anemones suggests that translocated compounds or their products were being directed to the urea cycle in the novel association (electronic supplementary material, figure S2). This is of particular interest given that nitrogen cycling within the symbiosis involves ammonium produced via the urea cycle [66–68], and that increased activity of the urea cycle may therefore enhance symbiont replication [69]. If this is the case, then this physiological response could facilitate growth of *D. trenchii* in our study (figure 2; electronic supplementary material, figure S2). By contrast, the absence of enrichment of ornithine and glutamine in the homologous symbiosis suggests a reduction in ammonium-producing pathways such as the urea cycle, which could act to limit symbiont growth and biomass, and prevent overgrowth of symbionts in host tissues [70–72]. The dissimilar physiological responses to the different symbiont types suggest that the algal symbiont alters host metabolism beyond the provision of fixed carbon.

(iii) Antioxidant composition
Both partners of the cnidarian–dinoflagellate symbiosis express a diverse repertoire of enzymatic and non-enzymatic antioxidants to prevent oxidative damage [73–76]. Multiple antioxidant metabolites and precursors were labelled in both the homologous and heterologous symbiotic states, including mannitol and glutamate (a glutathione precursor). This is consistent with the production of antioxidants by Symbiodiniaceae and the expression of a greater diversity and activity of antioxidants when in symbiosis [73,77]. Mannitol was labelled in both associations, but *B. minutum*-colonized hosts showed a 5- and 10-fold increase in the abundance of labelled and unlabelled pools, respectively, relative to hosts containing *D. trenchii*. In algae, mannitol functions in osmoregulation, sugar storage and non-enzymatic scavenging of hydroxyl radicals [78]. Many plant pathogens have the ability to synthesize mannitol, and there is growing evidence that phytopathogenic fungi use mannitol to suppress reactive oxygen species-mediated defences in the host [79]. Mannitol translocation may provide a mechanism for avoiding host defence mechanisms to an invading organism via redox homeostasis in the establishment and maintenance of the cnidarian–dinoflagellate symbiosis [18,80,81]. Alternatively, the translocation of antioxidant metabolites is a potential mechanism of thermo- and oxidative-tolerance afforded to the holobiont by the Symbiodiniaceae cells [82], and different taxa may provide different amounts or varieties of antioxidants [26]. Indeed, greater thermal and oxidative-tolerance is attributed to *D. trenchii* [11,28] and may be linked to the photoprotective and antioxidant compound repertoire of *Durusdinium* [83]. Therefore, the antioxidant capabilities of *D. trenchii* may explain its ability to form novel associations [18].

(e) Metabolic flux and the fate of the symbiosis
Both symbiotic partners can influence the fate of a novel association, the host by the intensity of its immune response and the symbiont by the degree of physiological perturbations inflicted on the host [18,84]. The differences in nutritional exchange that we report here have the potential to play a central role in such responses, either through inter-partner signalling or downstream effects on host or symbiont physiology (figure 3). In particular, our results suggest that, if a novel symbiosis is unable to adjust its physiology to compensate for qualitative differences in its metabolite pools (e.g. via heterotrophic compensation [23,43,45]), then its fitness and survivorship could be compromised. Our results define some of the nutritional trade-offs for cnidarians that associate with less competent symbionts. Such information is of significant value when predicting the long-term success of stress-driven symbioses, and will improve our ability to model and predict the future of coral reef systems under climate change scenarios. Further investigation, using more Symbiodiniaceae taxa spanning a variety of physiological traits, will help to shed further light on this matter, as will the identification of more metabolites involved in the symbiosis. Metabolic flux analysis of metabolites passing from alga to host and vice versa, combined with visualization of the cellular fate of labelled compounds in the host tissues and symbiont cells, will provide a means to understanding the role played by mutual nutritional exchange in the formation of novel symbiotic associations and the capacity of reef corals to adapt to climate change through switching partners.

References


Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

Authors’ contributions. J.L.M. carried out the laboratory work, participated in the data analysis, carried out the statistical analyses, participated in the design of the study and drafted the manuscript; C.A.O. participated in the design of the study and helped draft the manuscript; A.L. participated in the data analysis; K.E.H. participated in the data analysis; U.R. participated in the data analysis; A.R.G. participated in the design of the study and helped draft the manuscript; V.M.W participated in the design of the study and helped draft the manuscript; S.K.D. participated in the design of the study and helped draft the manuscript. All authors gave final approval for publication.

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