Competitive traits of coral symbionts may alter the structure and function of the microbiome

Shelby E. McIlroy 1,2 · Jane C. Y. Wong 1,2 · David M. Baker 1,2

Received: 6 November 2019 / Revised: 6 May 2020 / Accepted: 14 May 2020 / Published online: 9 June 2020 © The Author(s) 2020. This article is published with open access

Abstract
In the face of global warming and unprecedented coral bleaching, a new avenue of research is focused on relatively rare algal symbionts and their ability to confer thermal tolerance to their host by association. Yet, thermal tolerance is just one of many physiological attributes inherent to the diversity of symbiodinians, a result of millions of years of competition and niche partitioning. Here, we revealed that competition among cocultured symbiodinians alters nutrient assimilation and compound production with species-specific responses. For Cladocopium goreaui, a species ubiquitous within stable coral associations, temperature stress increased sensitivity to competition eliciting a shift toward investment in cell replication, i.e., putative niche exploitation. Meanwhile, competition led Durusdinium trenchii, a thermally tolerant “background” symbiodinian, to divert resources from immediate growth to storage. As such, competition may be driving the dominance of C. goreaui outside of temperature stress, the destabilization of symbioses under thermal stress, the repopulation of coral tissues by D. trenchii following bleaching, and ultimately undermine the efficacy of symbiont turnover as an adaptive mechanism.

Introduction
In marine phytoplankton, interspecific variability in metabolic responses to light, temperature, and nutrients are important drivers of competition and community assembly [1–3]. Indeed, constraints on growth by limiting nutrients such as nitrogen and phosphorus can have profound implications for species dominance in the surface ocean, and downstream consequences for ecosystem functioning. For example, Burson et al. [2] showed that variation in nitrogen and phosphorus loads could induce single or co-limitation of resources (both nutrients and light), which altered the community composition of phytoplankton. Nitrogen in particular is broadly limiting to life on Earth as it is the fundamental building block for amino and nucleic acids—both critical for cell division and growth. It is therefore curious that despite decades of accumulated knowledge on the impact of nutrients on coral reef ecosystems, there have been few studies to examine the role of nutrient competition on in hospite communities of dinoflagellates.

Endosymbiotic dinoflagellates from the genetically diverse lineage Symbiodiniaceae [4] supply the majority of a coral’s energy (carbon; [5]) and growth (nitrogen; [6, 7]) resources through photosynthesis and tight recycling of the host’s metabolic waste. Indeed, emerging evidence suggests that host modulation of nitrogen to the symbionts keeps them in a nitrogen-limited state [8]—a condition which is well-known to slow cell division and induce the accumulation of carbohydrates and lipid compounds with relatively high C:N ratios in marine phytoplankton [9, 10]. While coral species/individuals tend to associate with one or a few predictable, dominant symbionts, these associations can vary with depth (light availability) and temperature. In hospite distributions of symbiodiniain species along light gradients optimize interspecific variability in photosynthetic performance [11, 12], with niche partitioning along micro-scales [13, 14] and macro-scales [15]. Meanwhile, some

These authors contributed equally: Shelby E. McIlroy, Jane C. Y. Wong

Supplementary information The online version of this article (https://doi.org/10.1038/s41396-020-0697-0) contains supplementary material, which is available to authorized users.

David M. Baker
dmbaker@hku.hk

1 The Swire Institute of Marine Science, The University of Hong Kong, Hong Kong, Hong Kong, PRC
2 School of Biological Sciences, The University of Hong Kong, Hong Kong, Hong Kong, PRC
species persist at background levels while providing no discernible function ([16]; but see [17]). It is these background types, generally within the genus Durusdinium, which transition in and out of dominance following disturbance [18]. Interestingly, these opportunists are often associated with thermal tolerance [19]. While variation in functional traits of symbionts is clearly an important determinant of holobiont physiology [20], it can also drive the outcomes of ecological interactions among those microbes, and the subsequent efficacy of microbial turnover as an acclimatory mechanism. This raises an important question: is competition among symbionts limiting the proliferation of these thermally tolerant species in an ever-warming world, and in particular, how do the different symbiodiniants compete?

One of the reasons that ecological interactions among microbes are still poorly understood is the difficulty in isolating the function of specific microbes within a complex microbiome [21]. To understand competition between Symbiodiniaceae species, we traced the assimilation of isotopically enriched carbon and nitrogen in cultures of two common co-associates of Acropora spp. (Cladocetiopsis goreaui and Durusdinium trenchii). Where C. goreaui and D. trenchii were grown together within the same flask (i.e., mixed species cultures), we used a combination of species-specific fluorescent in situ hybridization (FISH) labeling and flow cytometric sorting to separate cells of each species (Flow) with subsequent stable isotope analysis (SIA). We hypothesized that the carbon and nitrogen assimilation and resulting changes in their stoichiometry (C:N) would be distinct for each species in ways that could underpin their ecological distributions, and that these metabolic traits could be modified in response to the presence of a competitor. To assess this, the FISH-Flow-SIA methodology was applied to a fully crossed experimental design with temperature (26 and 32 °C) and interspecific competition (present and absent) as factors.

Materials and methods

Culture isotope enrichment pulse

Cultures of C. goreaui and D. trenchii were acquired from the Symbiont Culture Facility at the Australian Institute of Marine Science (see Table S1 for details). Cultures were subsequently maintained in batch culture at 26 °C and 120 µmol photons m⁻² s⁻¹ with monthly transfers into 0.22 µm filtered artificial seawater (Instant Ocean Spectrum Brands) supplemented with f/2 media (Gillard 1975). For isotopically enriched pulses, f/2 media was amended with Na¹³CO₃ (98% ¹³C, Sigma; [H¹²CO₃⁻] = 1.18 mM) and Na¹⁵NO₃ (98% ¹⁵N, Sigma; [NO₃⁻] = 3.88 µM). To each flask we added 100 mL of enriched media and ~10,000,000 total cells per flask among the following treatments: C. goreaui monoculture (n = 8), D. trenchii monoculture (n = 8), C. goreaui + D. trenchii (0.5:0.5) coculture (n = 16). The increase in coculture replicates was in anticipation of potential data loss in splitting the sample for species-specific labeling, which can result in too low of sample mass for SIA analyses. Furthermore, two of the coculture replicates were analyzed in bulk (without FISH-Flow) for methods development (ESM Appendix 1; Fig. S3). After 4 h of incubation under 200 µmol photons m⁻² s⁻¹ at 26 or 32 °C, samples were processed in batches under low light and in a random order to limit any bias of additional nutrient assimilation during sample processing. We transferred cells to 50 mL falcon tubes, centrifuged at 5000 × g for 5 min, washed twice with deionized water, and fixed with 5X SET Buffer (3.75 mol l⁻¹ NaCl, 25 mmol l⁻¹ Na₂EDTA, 0.5 mol l⁻¹ Tris, pH 7.8). Samples were then stored at −80 °C until further analyses. Experimental light level was chosen to avoid light limitation and was confirmed with rapid light curves on individual cultures to be below potential stress levels (ESM Fig. S5).

FISH protocol

Samples were treated with a modified protocol from [22]. Immediately preceding hybridization, samples were thawed and washed sequentially in 5X SET with IGEPAL at 0.4 and 0.1% vol/vol. Samples were split among four tubes to decrease cell concentrations and increase probe efficiency. For hybridization, samples were incubated overnight at 45 °C in hybridization buffer (5X SET, 0.1% vol/vol IGEPAL, 10% vol/vol formamide) with 100 pmol of a probe. C. goreaui monoculture and D. trenchii monoculture were hybridized with SymC and SymD [22], respectively, while C. goreaui + D. trenchii coculture treatments were split and hybridized with SymC and SymD separately. The following morning, samples were centrifuged and washed with warm 1X SET for 5 min, vortexed and resuspended in 500 ul of 1X SET at room temperature, and subjected to cytometric analysis within 1–5 h.

Flow cytometry and sorting

All samples were analyzed and sorted on a BD Influx (BD Biosciences, San Jose, CA). ACCUDROP beads (BD Biosciences) were used to set drop delay at the start of each session. A gating hierarchy was used to set the conditions for sorting samples. First a gate was used to screen for cells of the appropriate shape and size (FSC-A vs. SSC-A; Fig. S1a), then two gates were used to remove doublets (FSC-W vs. FSC-H and SSC-W vs. SSC-H; Fig. S1b, c). Gating of probe-positive cells within a treatment was
dependent on their position within the 582 nm vs. SSC-A (Fig. S1d, e); the gated cells were sorted using two-way yield mode.

### Stable isotope analysis

The sorted cells were rinsed twice with DI water and harvested by centrifugation (4000 x g, 5 min, 4 °C). The resulting pellets were transferred to pre-tarred tin capsule and oven dried at 60 °C overnight. To ensure that N masses were above the detection limit for reliable δ¹⁵N values, 0.18 mg of nitrogen carrier (=42.6 µM of NaNO₃) was added to each sample before analysis [23]. Samples were combusted in an elemental analyzer (Eurovector EA3028) coupled to a stable isotope ratio mass spectrometer (Nu Instruments Perspective) in continuous flow mode. Internal standards (acetanilide; Indiana University, IN, USA) were measured to normalized sample δ¹⁵N and δ¹³C values. Mean precision for δ¹⁵N and δ¹³C were 0.25‰ and 0.07‰, respectively. Isotopic enrichment was corrected to account for dilution by the carrier. Due to uncertainty in developing novel methodologies, data occasionally fell outside of the range of detection because either not enough sample mass was obtained for ¹⁵N readings, or the ¹³C signal was oversaturated due to user error in helium dilution settings. The resulting sample sizes are reported in Table 1.

Detailed methodological development and validations of FISH-Flow-SIA are provided in ESM Appendix 1 and Figs. S1–S4.

### Statistics

All analyses were run in base R version 3.5.1 (R Core Team 2018). Analysis of variance was used to determine the response of variables to fixed factors as outlined below. For each test, assumptions of ANOVA were tested. Hedge’s g was used to report effect sizes to account for variation in sample sizes.

#### Effects of methodology on stable isotope readings

A one-way ANOVA was used to compare atom percent (AP) values of ¹³C and ¹⁵N of samples collected and analyzed directly after experiment and those subjected to FISH labeling and flow cytometry (see Appendix S1).

#### Functional differences

Data from monocultures of *C. goreaui* and *D. trenchii* were included in a two-factor ANOVA with species and temperature as fixed factors and response variable of either AP ¹³C or AP ¹⁵N.

#### Competition

To understand the effect of competition on AP ¹³C and AP ¹⁵N, we subset the data for each species and ran a two-factor ANOVA on each dataset using temperature and competition (presence/absence) as fixed factors.

#### Stoichiometry of response

The ¹³C to ¹⁵N ratio was calculated for each sample. After subsetting the data for each species, the effects of temperature and competition for each species were determined with ANOVA.

### Results

#### Assumptions of statistical tests

When testing the effect of temperature and competition on AP ¹⁵N for *C. goreaui*, we found a significant violation of homogeneity of variance (Levene’s test *p* = 0.002). Three outliers spread across treatments were identified. When these were excluded, the remaining dataset satisfied the assumption of homogeneity of variance (Levene’s test *p* = 0.39). Subsequent ANOVAs on this dataset with and without these outliers included resulted in the same statistical significance outcomes. Therefore, we retained the full dataset for statistical reporting and data plots. Despite variation in sample size among treatments (Table 1) all other statistical comparisons met the assumptions of the ANOVA.

### Table 1 Samples sizes for carbon and nitrogen analyses.

| Temperature (°C) | Competition | Species | n for AP ¹³C | n for AP ¹⁵N |
|-----------------|-------------|---------|--------------|--------------|
| 26              | Absent      | C.g.    | 8            | 8            |
| 26              | Present     | C.g.    | 14           | 14           |
| 32              | Absent      | C.g.    | 2            | 8            |
| 32              | Present     | C.g.    | 12           | 8            |
| 26              | Absent      | D.t.    | 7            | 8            |
| 26              | Present     | D.t.    | 13           | 13           |
| 32              | Absent      | D.t.    | 2            | 8            |
| 32              | Present     | D.t.    | 12           | 12           |

Experimental replicates for the experiment were *Cladocopium goreaui* (C.g.) monoculture (*n* = 8), *Durusdinium trenchii* (D.t.) monoculture (*n* = 8), *C. goreaui* + *D. trenchii* coculture (*n* = 14). However, due to uncertainty in developing novel methodologies, data occasionally fell outside of the range of detection because either not enough sample mass was obtained for ¹⁵N readings, or the ¹³C signal was oversaturated due to user error in helium dilution settings.
two-factor ANOVA on AP $^{13}$C data revealed a significant effect of temperature ($F_{(15,1)} = 6.68, p = 0.02$), and a significant temperature by species interaction ($F_{(15,1)} = 8.6, p = 0.01$; Fig. 1a). At the 26 °C baseline, carbon assimilation for both $C. goreaui$ and $D. trenchii$ was significantly higher than AP $^{15}$C in comparison with each of the other baseline treatments (Tukey’s HSD $p < 0.02$). For AP $^{15}$N, ANOVA revealed a significant temperature effect ($F_{(28,1)} = 22.4, p < 0.001$) and species ($F_{(28,1)} = 21.9, p < 0.001$) effect with no interaction ($p = 0.53$; Fig. 1b). At 26 °C, $D. trenchii$ had a higher rate of nitrogen assimilation (AP $^{15}$N 0.64 ± 0.06) relative to $C. goreaui$ (AP $^{15}$N 0.55 ± 0.04). The relative increase in nitrogen assimilation at 32 °C for $C. goreaui$ and $D. trenchii$ was 18% and 12%, respectively (Fig. 1b).

Interspecific competition

For $C. goreaui$, there was a significant effect of competition ($F_{(1,32)} = 5.6, p = 0.02$), temperature ($F_{(1,32)} = 53.7, p < 0.001$), and a competition × temperature interaction ($F_{(1,32)} = 8.2, p = 0.007$) on AP $^{15}$C (Fig. 1c). Post hoc comparisons revealed that carbon assimilation at 32 °C was significantly decreased in response to the presence of a competitor (Tukey’s HSD $p < 0.001$; Hedge’s $g = −3.84$, Fig. 1g). For AP $^{15}$N, there was a significant effect of competition ($F_{(1,34)} = 12.4, p = 0.001$), temperature ($F_{(1,34)} = 63.0, p < 0.001$), and a competition × temperature interaction ($F_{(1,34)} = 16.5, p < 0.001$; Fig. 1d). Post hoc comparisons showed that at 32 °C AP $^{15}$N in $C. goreaui$ was significantly higher when competition was present (Tukey’s HSD $p < 0.001$; Hedge’s $g = 2.17$, Fig. 1h). For $D. trenchii$, AP $^{15}$C was significantly affected by competition ($F_{(1,30)} = 10.5, p = 0.002$), but not temperature ($p = 0.83$), nor a significant interaction term ($p = 0.87$; Fig. 1c). Contrastingly, AP $^{15}$N was significantly affected by temperature ($F_{(1,37)} = 21.6, p < 0.001$), but not competition ($p = 0.43$), nor their interaction ($p = 0.11$; Fig. 1d).

Stoichiometry of the response

Overall, the changes in nutrient assimilation significantly affected the C:N ratio of newly assimilated compounds (Fig. 2). For $C. goreaui$, ANOVA revealed a significant effect of temperature ($F_{(1,28)} = 24.0, p < 0.001$) and competition ($F_{(1,28)} = 15.9, p < 0.001$), without a temperature ×
assimilated compounds (Fig. 2). Monocultures of *C. gor-eaui* and *D. trenchii* were used to establish species-specific differences in metabolism and their “baseline” response to increased temperature when grown in isolation (Fig. 1a, b). We then measured each species’ response to competition relative to those baselines. Specifically, *C. gor-eaui* exhibited traits of exploitative competition, increasing the proportion of nitrogen rich biomolecules—proteins and nucleic acids which are all essential for cell division [9], but only at increased temperature (Fig. 2a). Meanwhile, an increase in the relative proportion of newly assimilated carbon-rich biomolecules in *D. trenchii* when in the presence of *C. gor-eaui* is reflective of investments in carbohydrates and lipids for future growth ([9]; Fig. 2b). While herein limited to species in culture, these findings provide a foundation and a methodology for considering the role of competition in microbial community structure and success- sion *in hospite*.

**Functional variability**

The distribution of species within their environment, whether free living or *in hospite*, will be affected by species traits and their functional variability. Both light and temperature are known to affect *Symbiodiniaceae* distributions at both the free living and *in hospite* stages (e.g., [12, 26]). At the 26 °C baseline, carbon assimilation for both *C. gor-eaui* and *D. trenchii* was similar but *D. trenchii* had a higher rate for nitrogen. Increased temperature further dis- tinguished the metabolic strategy of the two species. *C. gor-eaui* increased both carbon and nitrogen assimilation at 32 °C, with a relatively small subsequent change in C:N (Fig. 2a). Documented effects of moderate temperature stress on *Symbiodiniaceae* include loss of photosynthetic membrane integrity (hours–days), buildup of reactive oxygen species (days), loss of photosynthetic function (days–weeks), and cell death (days–weeks) (reviewed in [27, 28]); each of these are associated with a decrease in nutrient assimilation. Thus, the punctuated increase in metabolic rate of *C. gor-eaui* in response to a temperature increase is unlikely driven by an impairment of photo- synthesis, particularly within such a short (4 h) timeframe. At higher temperatures *D. trenchii* increased nitrogen, but not carbon, assimilation, resulting in a relatively large decrease in C:N of newly assimilated compounds (7.0 vs. 5.7; Fig. 2b), a response known to precede cell division [9, 29, 30]. The differences in baseline nutrient demand demonstrated here not only provide insight into how these species respond to temperature, but given oligotrophic nature of tropical marine habitats, this functional variation in nutrient acquisition and assimilation can be another important axis along which niche partitioning may be taking place [31].

**Discussion**

Microbes compete in one of two ways: passive, exploitative competition, wherein one species consumes a limiting resource, restricting the availability of that resource to a competitor; and active, interference competition, in which individuals damage one another via antimicrobial secretions or contact-dependent killing [24]. Differentiating the two requires a clear accounting of the response of each individual competing species such as that made possible by FISH-Flow-SIA. Furthermore, environmental factors, such as temperature, can influence performance and a species’ ability to acquire resources and thus modulate competitive responses [3, 25]. In this study, we demonstrated that interspecific competition drives changes in the rates of carbon and nitrogen assimilation exhibited by two homologous *Symbiodiniaceae* species (Fig. 1a–d), which led to significant changes in the stoichiometry of newly
**Interspecific competition**

In this study, we found species-specific metabolic responses to the presence of competition that manifested within just 4 h of exposure. For example, in the presence of *C. goreaui*, *D. trenchii* significantly increased carbon assimilation under both temperature treatments. This drove a proportional shift toward newly assimilated compounds that were higher in C: N, a well-known response by algae to nitrogen limitation and evidence of a shift toward investments in storage compounds that would be beneficial when environmental and/or ecological conditions became more favorable (e.g., when released from competition; [9, 32]). Contrastingly the competitive response of *C. goreaui* was to increase nitrogen assimilation by 30% and reduce carbon assimilation by 14%, but this response was temperature specific occurring only at 32 °C (Fig. 1a, b). Thus, despite the increase in baseline metabolic demand observed in *C. goreaui* monocultures under thermal stress, competing *C. goreaui* invested proportionally more into lower C:N compounds, which suggests a boosted investment in cell replication. Similar shifts to increased growth rates as a mechanism for resource exploitation have been observed in many microbial systems in competition [24]. However, given the culture media conditions, the short (4-h) timeframe of our study, and the mismatch in species sensitivity to temperature it is unlikely that *C. goreaui* could have been effective in exploiting nitrogen to the point of limitation for *D. trenchii*. Instead of resource exploitation/limitation, the competitive response of *D. trenchii* may be the result of interference competition. The co-option of signaling molecules (i.e., micro-RNAs) and/or potential allelopathic compounds, which is widespread among dinoflagellates [33, 34], could instead be implicated and deserve further attention. In this case, however, the ability of *D. trenchii* to exhibit enhanced metabolism (carbon assimilation) under competition without trade-offs in nitrogen assimilation may be the key to its ability to persist at background levels [35, 36]. Interestingly, *C. goreaui* is the dominant symbiont type in many corals at ambient temperatures [37] and was not sensitive to interspecific competition at 26 °C (Fig. 1c).

**Implications for symbiosis**

As in every ecological system, competitive intensity and outcomes will be determined by environmental conditions. Thus, while experiments with symbionts in culture are an essential first step for understanding competitive potential among symbionts outside of host influence, the underlying mechanisms of competition both inside and outside of hosts require further investigation across various hosts and environmental conditions. The cultures used in this study were chosen for the fact that they can co-occur and transition in and out of dominance within a host [36]. At ambient temperatures (26–28 °C) corals that host predominantly *C. goreaui* receive more carbon [38] and nitrogen [39], and grow faster [40, 41], but are generally more sensitive to heat stress (30–32 °C) and bleaching relative to corals that host predominantly *D. trenchii* ([41, 42], but see [43]). The intrinsic differences between multiple species and how they respond to competition in culture can provide baseline expectations of how functional and ecological traits contribute to the ultimate composition of symbiont communities in hospite. For example, in N-limited coral reef habitats, symbionts with low nitrogen demand will have a relatively lower sensitivity to nutrient limitation and therefore higher competitive potential in oligotrophic environments [42]. Nitrogen limitation within coral cells has recently been confirmed by NanoSIMS with density dependent effects on symbiont populations [8]. The lower baseline requirement for nutrients observed in *C. goreaui* relative to *D. trenchii* at 26 °C correlates with their tendency to dominate coral hosts under ambient temperature conditions [19]. In response to thermal stress, increased respiration in *Cladocopium* species led to a shift toward parasitism in its relationship with host *Orbicella faveolata* [30] demonstrating that imbalances in holobiont nutrient recycling contribute to the destabilization of a healthy symbiotic state. In culture, the metabolic response of *C. goreaui* was especially pronounced where both temperature stress and competition were present which would further divert resources from the symbiosis. However, understanding how competitive traits translate to nutrient sharing in symbiosis will require the application of FISH-Flow-SIA to in hospite systems with simultaneous measures of host tissue enrichments at macro scales.

*D. trenchii*, confers increased temperature tolerance to some *Acropora spp.* and other coral hosts globally. Based on these patterns observed in hospite, we expected *D. trenchii* to be relatively insensitive to increased temperature [39] with a temperature-dependent response to competition. Instead, *D. trenchii*, assimilated more nitrogen at 32 °C and was consistently sensitive to competition across temperatures (Fig. 1c, d). Thus, rather than outcompeting *C. goreaui* in any scenario, it is more likely that *D. trenchii* is the “last man standing” as it is able to tolerate and then repopulate coral tissues under conditions unsuitable for other symbionts. In other words, this species seems well suited to proliferate only in the absence of other strains, e.g., under extreme environmental conditions [35], following bleaching [18], and in newly available host habitat [44]. Indeed, during the initial colonization of coral recruits *D. trenchii* often remained at lower than expected densities when other symbionts were present despite being able to fully colonize...
corals in their absence [44]. When temperature stress is removed, *D. trenchii* is driven to background levels by competitive dominance of other species, which replace *D. trenchii* over time [18]. Relative to *C. goreau*, *D. trenchii* showed higher baseline nitrogen assimilation at both temperatures which aligns with the increased cost and potential consequences demonstrated for corals of associating with thermally tolerant symbionts [38, 39].

Evidence of symbiont competition for host habitat [44] and resources [8] has been accumulating with potential negative effects on the coral host. The competitively driven changes in symbiobiont metabolism revealed herein would have downstream effects on the coral symbiosis potentially through both resource diversion, allelopathy, and/or competitive outcomes. Furthermore, variation in functional traits, and the pattern of dominance of *C. goreau* in corals at ambient temperatures and *D. trenchii* in corals subject to thermal stress, support the potential for competition to play a role in structuring *in hospite* communities. In the face of climate change, interventions to accelerate the adaptation of corals to withstand stress are warranted [45], with functional probiotics as a promising tool [46, 47]. However, if a microbial partner is not competitive across a range of ambient conditions, it will only occasionally achieve its desired function [48]. This presents a major obstacle to the efficacy of assisted evolution of mutualistic and stress resistant *Symbiodiniaceae*.

There is no shortage of interesting ecological and evolutionary questions that have arisen in parallel with the discovery of broad genetic diversity within microbial communities. However, isolating the function of specific microbes within a complex microbiome is challenging [21, 49]. We have demonstrated that established methodologies (FISH labeling, flow cytometry, and SIA) applied in a novel manner provides a new perspective into how competition among microbial symbionts may impact trajectories of holobiont health. The limitations of this technology to target a single species at a time are driven by the high auto fluorescence across a broad spectrum [50] will be overcome as fluorescent labeling technologies improve. The application of FISH-Flow-SIA to other taxonomic groups of microbes in corals and other hosts/environments requires only the redesign and validation of probe sequences for any target. Furthermore, probe design within FISH-Flow-SIA allows for flexibility in the taxonomic resolution of microbial targets and the ability to group taxa at higher or lower taxonomic levels. We believe that these methods and complementary emerging technologies (e.g., NanoSIMS combined with FISH labeling) will underpin a more comprehensive understanding of symbioses across systems, which includes revisiting not only the role of interspecific competition, but also a host’s ability to monopolize competitive outcomes through nutrient regulation.

**Data availability**

Raw data are available on GitHub repository at github.com/shelby26/FFS.

**Acknowledgements** We thank N. Knowlton, M.A. Coffroth, J. Mark Welch for feedback on the paper. Algal cultures were provided by the Symbiont Culture Facility at the Australian Institute of Marine Science; equipment was provided by The Stable Isotope Ratio Mass Spectrometry laboratory (SIRMS) at the University of Hong Kong and Li Ka Shing Faculty of Medicine Faculty Core Facility at The University of Hong Kong.

**Funding** Research Grants Council Hong Kong, GRF #17100014.

**Author contributions** SEM, JCYW, and DMB conceptualized the experiment, SEM and JCYW developed methodologies and carried out the experiment. All authors contributed significantly to the writing of the paper.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

**References**

1. Bestion E, García-Carreras B, Schaum C-E, Pawar S, Yvon-Durocher G. Metabolic traits predict the effects of warming on phytoplankton competition. Ecol Lett. 2018;21:655–64.
2. Burson A, Stomp M, Greenwell E, Grosse J, Huisman J. Competition for nutrients and light: testing advances in resource competition with a natural phytoplankton community. Ecology. 2018;99:1108–18.
3. Tilman D, Mattson M, Langer S. Competition and nutrient kinetics along a temperature gradient: an experimental test of a mechanistic approach to niche theory. Limnol Oceanogr. 1981:26:1020–33.
4. LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, et al. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr Biol. 2018;28:2570–80.e6.
5. Muscatine L, McCloskey LR, Marian RE. Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol Oceanogr. 1981:26:601–11.
Competitive traits of coral symbionts may alter the structure and function of the microbiome

6. Tanaka Y, Suzuki A, SAKAI K. The stoichiometry of coral-dinoflagellate symbioses: carbon and nitrogen cycles are balanced in the recycling and double translocation system. ISME J. 2018;12:860–8.

7. Pernice M, Meibom A, Van Den Heuvel A, Kopp C, Domart-Coulon I, Hoegh-Guldberg O, et al. A single-cell view of ammonium assimilation in coral-dinoflagellate symbiosis. ISME J. 2012;6:1314–24.

8. Krueger T, Horwitz N, Bodin J, Giovanni M-E, Escrig S, Fine M, et al. Intracellular competition for nitrogen controls dinoflagellate population density in corals. Proc Biol Sci. 2020;287:20200049.

9. Liefer JD, Garg A, Fyle MH, Irwin AJ, Benner I, Brown CM, et al. The macromolecular basis of phytoplankton C:N:P under nitrogen starvation. Front Microbiol. 2019;10:763.

10. Talmy D, Blackford J, Hardman-Mountford NJ, Polimene L, Follows MJ, Geider RJ. Flexible C:N ratio enhances metabolism of large phytoplankton when resource supply is intermittent. Biogeosciences. 2014;11:4881–95.

11. Iglesias-Prieto R, Beltran VH, LaJeunesse TC, Reyez-Bonilla H, Thomé PE. Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. Proc Biol Sci. 2004;271:1757–63.

12. Kemp DW, Hernández-Pech X, Iglesias-Prieto R, Fitt WK, Schmidt GW. Community dynamics and physiology of Symbiodinium spp. before, during, and after a coral bleaching event. Limnol Oceanogr. 2014;59:788–97.

13. Rowan R, Knowlton N, Baker A, Jara J. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature. 1997;388:265–9.

14. Wangpraseurt D, Larkum AWD, Ralph PJ, Kühl M. Light gradients and optical microchirons in coral tissues. Front Microbiol. 2012;3:316.

15. Finney JC, Pettay DT, Sampayo EM, Warner ME, Oxenford HA, LaJeunesse TC. The relative significance of host-habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus Symbiodinium. Micro Ecol. 2010;60:250–63.

16. Lee MJ, Jeong HJ, Jang SH, Lee SY, Kang NS, Lee KH, et al. Most low-abundance ‘background’ Symbiodinium spp. are transitory and have minimal functional significance for symbiotic corals. Micro Ecol. 2016;71:771–83.

17. Ziegler M, Eguluiz VM, Duarte CM, Voolstra CR. Rare symbionts may contribute to the resilience of coral-algal assemblages. ISME J. 2018;12:161–72.

18. Thornhill D, LaJeunesse T, Kemp D, Fitt W, Schmidt G. Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. Mar Biol. 2006;148:711–22.

19. Baker A, Starger C, McClanahan T, Glynn P. Corals' adaptive response to climate change. Nature. 2004;430:741.

20. Sogin EM, Putnam HM, Nelson CE, Anderson P, Gates RD. Correspondence of coral holobiont metabolome with symbiotic bacteria, archaea and Symbiodinium communities. Environ Microbiol Rep. 2017;9:310–5.

21. Tropini C, Earle KA, Huang KC, Sonnenburg JL. The gut microbiome: connecting spatial organization to function. Cell Host Microbe. 2017;21:433–42.

22. McLrroy SE, Smith GJ, Geller JB. FISH-Flow: a quantitative molecular approach for describing mixed clade communities of Symbiodinium. Coral Reefs. 2014;33:157–67.

23. Kang Y, Kudela RM, Gobler CJ. Quantifying nitrogen assimilation rates of individual phytoplankton species and plankton groups during harmful algal blooms via sorting flow cytometry. Limnol Oceanogr Methods. 2017;15:706–21.

24. Ghoul M, Mitrí S. The ecology and evolution of microbial competition. Trends Microbiol. 2016;24:833–45.
45. Van Oppen MJH, Oliver JK, Putnam HM, Gates RD. Building coral reef resilience through assisted evolution. Proc Natl Acad Sci. 2015;112:2307–13.
46. Morgans CA, Hung JY, Bourne DG, Quigley KM. Symbiodiniaceae probiotics for use in bleaching recovery. Restor Ecol. 2020;28:282–8.
47. Peixoto RS, Rosado PM, de Assis Leite DC, Rosado AS, Bourne DG. Beneficial Microorganisms for Corals (BMC): proposed mechanisms for coral health and resilience. Front Microbiol. 20107;8. e-pub ahead of print. https://doi.org/10.3389/fmicb.2017.00341.
48. Bauer MA, Kainz K, Carmona-Gutierrez D, Madeo F. Microbial wars: competition in ecological niches and within the microbiome. Micro Cell. 2018;5:215–9.
49. Douglas AE. How multi-partner endosymbioses function. Nat Rev Microbiol. 2016;14:731–43.
50. Ainsworth TD, Fine M, Blackall LL, Hoegh-Guldberg O. Fluorescence in situ hybridization and spectral imaging of coral-associated bacterial communities. Appl Environ Micro. 2006;72:3016–20.