ECOLOGY

Response to Comment on Trophic strategy and bleaching resistance in reef-building corals

Inga E. Conti-Jerpe[1]*, Philip D. Thompson[1], Cheong Wai Martin Wong[1], Nara L. Oliveira[2], Nicolas N. Duprey[1†], Molly A. Moynihan[3,4], David M. Baker[1]*

Recently, we published a novel method used to assess the trophic niches of different coral species and demonstrated that their nutrition varied considerably, with some species highly dependent on their photosynthetic algal symbionts and others able to feed on plankton to meet energetic requirements. Adjustments to the use of this tool are necessary when it is applied to other scientific questions and symbiotic organisms. We respond to a comment highlighting a risk of bias in the methods, discuss suggested adjustments, and propose further refinements to improve method robustness.

Thibault and colleagues provide thoughtful insights on the application of Stable Isotope Bayesian Ellipses in R (SIBER) analysis (*1*) to paired coral host and algal symbiont stable isotope data, first presented in our paper "Trophic strategy and bleaching resistance in reef-building corals" (*2*). They identified three considerations and made three suggestions when using this method to investigate coral trophic strategy. We agree with many of Thibault *et al.*'s arguments and emphasize that our use of SIBER to assess mutualism is novel and may require further refinement. However, we question whether some of their suggestions are broadly applicable and argue that this new application of SIBER must be tailored to the specific questions and hypotheses under investigation.

Thibault *et al.* correctly highlighted that the stable isotope values of corals and their symbionts are affected primarily by variations in the environmental sources of carbon and nitrogen assimilated, as well as any fractionations associated with uptake (*3*). In our study system, we have a very good understanding of source stable isotope values and their spatiotemporal variation (*4*–*6*). Although we have confidence in our source fingerprinting, our method is further strengthened by paired sampling; host and symbiont fractions from the same holobiont inherently originate from the same environmental baseline. The use of paired host and symbiont data ensures that the sampling effort across space and time is the same for the two groups (host and symbiont), and thus eliminates baseline variation as a concern when applying SIBER to physically intertwined mutualisms.

Corals and their associated algae maintain an endosymbiosis where the algae cell lives within the cell membrane of their hosts, providing photosynthetic products for host nutrition. Thibault *et al.* infer from this that the maximum difference between the nitrogen stable isotope values of these two partners must therefore be 3.4‰, or an average trophic step seen between consumers and their dietary sources (*7*). Species-specific trophic discrimination factors, however, can vary from the mean (*8*), and in corals, applying a general value is even more tenuous due to their endosymbiosis (effectively a closed system) and degrees of resource sharing (*9*). Furthermore, Symbiodiniaceae are primary producers that can assimilate nitrate from the surrounding environment, whereas corals consume zooplankton that may be several trophic levels higher than phytoplankton at the base of the food chain, introducing even more potential for isotopic differences above 3.4‰. Thibault *et al.* argue that a limit on the maximum difference between host and symbiont increases the risk of SIBER analysis obscuring biologically relevant subgroups that exhibit autotrophy or mixotrophy within a larger dataset that appears heterotrophic overall. The fact that larger differences between host and symbiont $\delta^{15}N$ can and do occur demonstrates that there are situations where this risk is minimized. For instance, *Oulastrea crispata* collected from sites across Hong Kong showed an average difference between host and symbiont of 4.3 ± 2.3‰.

Thibault *et al.* are nonetheless correct that there is potential for our method to miss important variation—such as individual corals that may be exhibiting extreme autotrophy or heterotrophy relative to their conspecifics. Fox *et al.* (*10*) showed that it is possible for a minority of individuals to exhibit trophic strategies different from that of the majority, potentially in response to subtle variations in microhabitats or individual health. Thibault *et al.* suggest sampling at the highest spatial and temporal resolution to avoid missing these subgroups; however, sampling sufficiently for SIBER analysis at the smallest spatial scales is often not practical or even possible. Sample sizes of 30 or more are required to minimize the effect of sample size on ellipse area (*11*). Rather than constraining sampling areas, we suggest that investigators inspect the difference between host and symbiont isotope values (particularly $\delta^{15}N$) of each individual colony (*2*, *12*). These data can serve as an indicator of whether a subset of individuals potentially forms a group with a high amount of overlap between host and symbiont ellipses. Furthermore, the spatial scale of sampling is dependent on the scientific question being asked—in the case of our study, we were interested in capturing any propensity for heterotrophy under any environmental condition. While it is true that species may shift strategies across conditions, sampling across a wide environmental gradient was meant to reflect this shift where present. Sampling at a small spatial scale could miss trophic plasticity, but may be relevant in answering other questions about trophic strategy.

[1]The Swire Institute of Marine Science and the School of Biological Sciences, University of Hong Kong, Hong Kong Special Administrative Region, People's Republic of China. [2]Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, BA CEP 45640-000, Brazil. [3]Earth Observatory of Singapore, Interdisciplinary Graduate School, Nanyang Technological University, Singapore, Singapore. [4]Asian School of the Environment, Nanyang Technological University, Singapore, Singapore. *Corresponding author. Email: ingacontijerpe@gmail.com (I.E.C.-J.); dmbaker@hku.hk (D.M.B.)
†Present address: Max Planck Institute for Chemistry (Otto Hahn Institute), Hahn-Meitner-Weg 1, 55128 Mainz, Germany.

SIBER, as applied to symbiotic associations, requires a critical assessment of the ellipse area used with careful attention to statistical power. The standard ellipse area corrected for sample size (SEA_C) used by Jackson *et al.* (*1*) was designed to encompass one SD on either side of the mean of each plotted variable (*13*). In comparison, a 95% ellipse is fitted to a ±2.5 SD range. Thibault *et al.* suggest applying 95% ellipses when assessing the trophic strategy of symbioses to compensate for masked variation in seemingly heterotrophic populations drawn from heterogeneous environments. Smaller ellipse areas tend to exclude values and will indeed increase the likelihood of detecting heterotrophy, whereas larger ellipse areas tend to extrapolate values that may not be ecologically relevant (i.e., exceeding the stable isotope values of environmental sources) and will increase the likelihood of detecting mixotrophy or autotrophy. These are emblematic of committing type I and type II errors, respectively. We propose applying both of these ellipse sizes in reciprocal fashion to paired host and symbiont isotope data, using the 40% ellipse to test the hypothesis that a holobiont is autotrophic, and verifying that it is NOT heterotrophic with a 95% ellipse. Together, these are conservative tests that would raise the threshold for identifying an obligate autotroph or heterotroph and increase the probability of identifying mixotrophy without introducing a bias that corals are inherently mixotrophic.

Conti-Jerpe *et al.* (*2*) used the proportion of host SEA_C overlapping that of associated symbionts as an indicator of trophic strategy because this represents the proportion of the host trophic niche met through syntrophic resources. Thibault *et al.* proposed using overlap as a proportion of nonoverlapping area to assess trophic strategy, but do not justify this choice biologically or ecologically. Rather, they indicate that combining this metric with their other suggested alterations will result in more mixotrophic classifications. While additional metrics including the one proposed by Thibault *et al.* may be useful to estimate different aspects of nutrient sharing within mutualisms, we encourage users to interpret these metrics in a biological and ecological context.

Last, Thibault *et al.* asked what if coral hosts have lower nitrogen isotope values than their symbionts? It is important to consider how this will affect overlap metrics; however, as long as the isotope values of sources are different, the method will be robust. For instance, preliminary results from oligotrophic sites with episodic upwelling events (Dongsha Atoll, Taiwan and the Myeik Archipelago, Myanmar) revealed instances where hosts have lower $\delta^{15}N$ values than their symbionts. We believe that this is evidence of mixotrophy or heterotrophy best explained by hosts assimilating low $\delta^{15}N$ particulate organic matter (POM) produced from surface processes, whereas the symbionts are using upwelled dissolved inorganic nitrogen (DIN), which has a higher $\delta^{15}N$ value. Despite this different dynamic, we observed a similar pattern in the relative amount of host niche overlap across species, with *Acropora* exhibiting the most overlap, and *Turbinaria* and *Favites* exhibiting almost none. It is compelling that this method is revealing consistent results across disparate locations.

Thibault *et al.* raised important concerns about applying SIBER to paired symbiotic partners. We agree with their proposal to consider 95% ellipses when evaluating the trophic strategy of coral holobionts and suggest assessing this large ellipse along with the SEA_C to control for over- and underestimating ellipse overlap, respectively. We further support the use of different overlap metrics to estimate aspects of nutrient sharing within the holobiont as long as they are contextualized biologically. Last, we disagree with the assertion that sampling should always be conducted on the smallest spatial scale to control for corals' trophic plasticity; rather, the sampling design should reflect the scientific question and hypotheses under investigation. The stated objective of Conti-Jerpe *et al.* (*2*) was to estimate the trophic niches of coral genera. Inherently, niche definition generalizes trends observed across groups rather than exploring intraspecific variation. We therefore feel that our sampling design was not only appropriate for this objective but also a strength in that it included trophic flexibility across environmental conditions in our niche estimates. Our data clearly show a gradient of trophic flexibility, and these conclusions were supported by source isotope values as well as the difference between host and symbiont pairs. Nevertheless, discussing the considerations brought up by Thibault *et al.* will enable future studies to robustly apply this novel method to their studies on syntrophic interactions.

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Acknowledgments: We thank S. McIlroy and J. Cybulski for providing friendly reviews of the response manuscript. **Funding:** This work was supported by Research Grants Council Hong Kong Early Career 602 Scheme #789913 and Research Grants Council Hong Kong General Research Fund 603 #17100014. **Author contributions:** I.E.C.-J. and D.M.B. wrote the original draft. P.D.T., C.W.M.W., N.L.O., N.N.D., and M.A.M. reviewed and edited the manuscript. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data and code are available in the original paper (*2*).

Submitted 6 April 2021
Accepted 19 April 2021
Published 2 June 2021
10.1126/sciadv.abi8666

Citation: I. E. Conti-Jerpe, P. D. Thompson, C. W. M. Wong, N. L. Oliveira, N. N. Duprey, M. A. Moynihan, D. M. Baker, Response to Comment on [Trophic strategy and bleaching resistance in reef-building corals]. *Sci. Adv.* **7**, eabi8666 (2021).