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Coral holobiont research needs spatial analyses at the microbial scale

Madeleine J. H. van Oppen[1,2] | **Jean-Baptiste Raina**[3]

[1]Australian Institute of Marine Science, Townsville, Queensland, Australia

[2]School of BioSciences, The University of Melbourne, Parkville, Victoria, Australia

[3]Climate Change Cluster (C3), University of Technology Sydney, Sydney, New South Wales, Australia

Correspondence
Madeleine J. H. van Oppen, Australian Institute of Marine Science, PMB #3, Townsville, QLD 4810, Australia.
Email: m.vanoppen@aims.gov.au and madeleine.van@unimelb.edu.au

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A BRIEF HISTORY OF CORAL-MICROBE SYMBIOSIS RESEARCH

Historically, coral research has had a strong microbiological focus because corals engage in symbioses not only with prokaryotes, viruses and fungi—microbes found in association with most animals—but also with unicellular photosymbionts in the family Symbiodiniaceae (Dinophyceae). Up until the early 2000s, most of the coral microbiology literature focused on Symbiodiniaceae, aiming to understand the establishment of their symbiosis with corals, their contribution to the coral energy budget, their genetic and physiological diversity and the breakdown of this symbiosis resulting in the loss of the Symbiodiniaceae from the coral tissues. We now know that so long as *in hospite* Symbiodiniaceae are nitrogen-limited, they cannot use much of their photosynthetically fixed carbon for their own growth and instead translocate most photosynthate to the coral host, as such fulfilling most of the coral's nutritional requirements. This symbiosis underpins the ecological success of reef-building corals and allows these organisms to thrive in the nutrient-poor waters that are characteristic of the tropics.

The past 20 years of coral microbiology research have strongly emphasized the importance of other microbial associates in coral health and functioning. Much research effort today is directed at understanding the diversity and function of coral-associated bacteria and to a lesser extent that of other groups including archaea, fungi, viruses and filamentous algae that are found in the coral skeleton. However, in-depth knowledge of the exact function of individual microbial symbionts other than Symbiodiniaceae is limited to a few taxa and a handful of functions (e.g., Ceh et al., 2013; Kuek et al., 2022; Neave et al., 2016; Ritchie, 2006). Deciphering microbial function has been impeded by the huge taxonomic diversity of both scleractinian corals and their associated microbial communities. In addition, microbial communities exhibit considerable spatio-temporal variability among coral species, conspecific colonies and even within the same colonies. One way to address this challenge and untangle the function of individual microbial species is to conduct studies at scales that match the activity and ecology of these microorganisms.

Despite being classified as basal metazoans, corals are much more spatially complex than previously anticipated (Figure 1), with at least 37 transcriptionally distinct cell types present in adult corals (Levy et al., 2021). These cell types are grouped into microenvironments ('compartments'; i.e., coral mucus, ectodermis, gastrodermis, skeleton and gastrovascular cavity). Each compartment harbours specific microbes (e.g., Symbiodiniaceae occur in the gastroderm, fungi primarily in the skeleton, *Ostreobium* only in skeleton, different bacterial communities are found in mucus, tissue, gastrovascular cavity; Apprill et al., 2016; Hughes et al., 2022; Kimes et al., 2010; Pollock et al., 2018) and microbial community structure can even exist

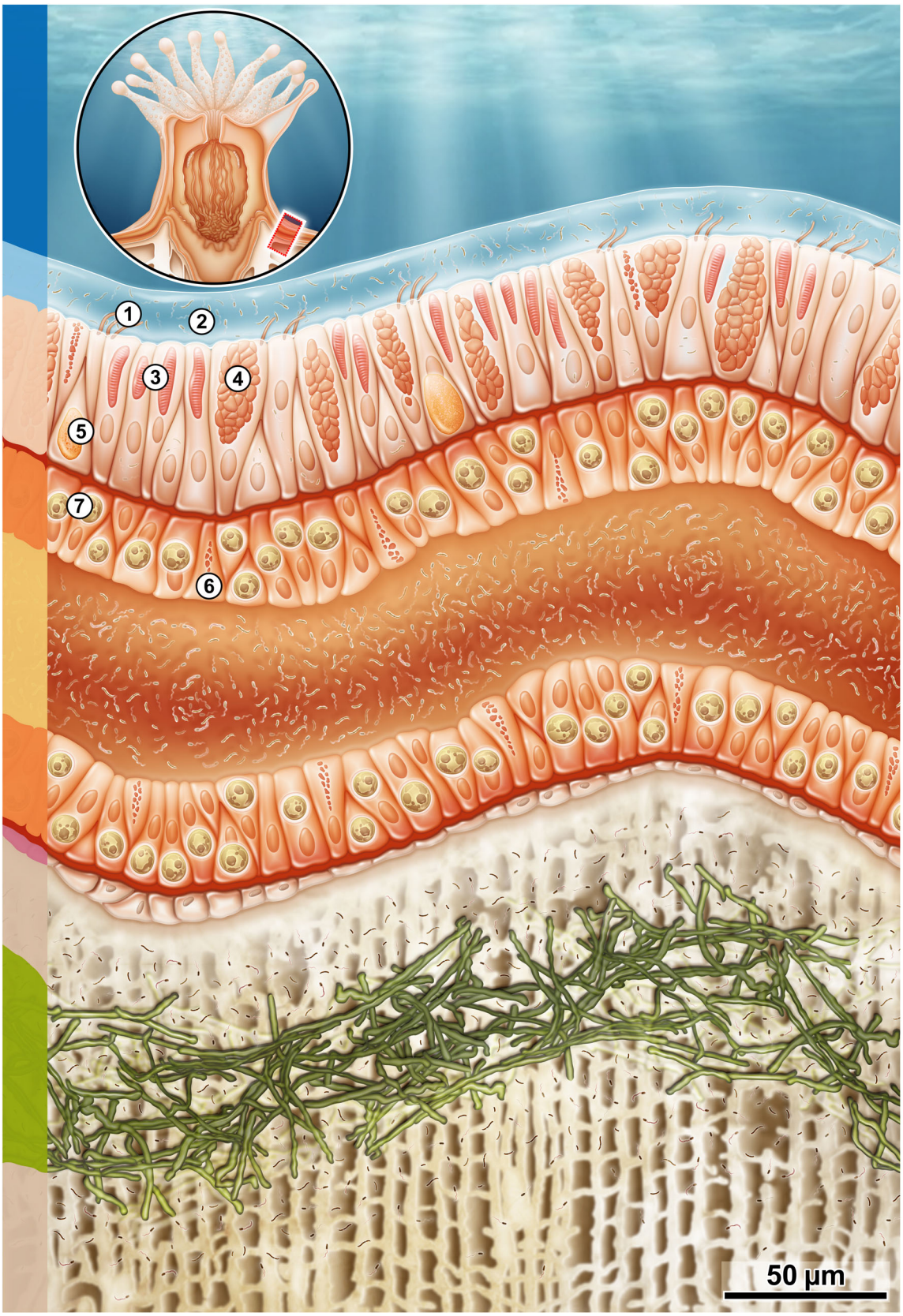

FIGURE 1 Legend on next page.

within a compartment (Marcelino et al., 2018). In fact, we know from other systems that microbial communities can be highly structured at the microscale. However, the most common approach to study coral-associated microbes (e.g., genotyping, amplicon sequencing, or various omics techniques) involves the homogenization of coral fragments. Similarly, a much-used method for photophysiological analysis of Symbiodiniaceae, pulse amplitude modulation fluorometry, is mostly conducted on cell populations rather than individual cells. If we were to study the complex interactions occurring between trees and insects in a forest, the bulk-scale approaches used in coral research would be analogous to grinding up the entire forest ecosystem. To a large extent, the use of these bulk approaches is due to the limited availability of methods to examine host-associated microbes at greater spatial resolution for non-model systems. However, to advance the field of coral microbial ecology, spatial approaches at the micrometre scale need to be embraced.

EXISTING SPATIAL STUDIES HAVE REVEALED IMPORTANT INSIGHTS INTO CORAL-MICROBE INTERACTIONS

Despite the predominant use of bulk analyses, a suite of spatial methods and analyses have been applied to corals, the earliest of which is the examination of histological sections with light microscopy. Even with simple stains, such as haematoxylin and eosin, the location of individual Symbiodiniaceae cells within the host gastrodermal cells and clusters of bacteria within the gastro- and ectodermis can be easily determined. Higher resolution microscopy, such as scanning (SEM) or transmission electron microscopy (TEM), can reveal the presence and location of individual bacteria, virus-like particles and other microbes. Nevertheless, these techniques cannot unambiguously discriminate intracellular prokaryotes and precisely identify the taxonomy of these microbes. The use of taxon-specific fluorescence in situ hybridization (FISH) probes coupled with epi-fluorescence or confocal microscopy can reveal the spatial organization of complex microbial assemblages, which has been critical to understanding interactions between different taxa in other hosts (e.g., Welch et al., 2016). Among the first to use FISH in corals were Bythell et al. (2002) who demonstrated the presence of coccoid bacterial cells in disease lesions of the Caribbean coral *Acropora palmata*, and Lesser et al. (2004) who applied a specific 16S rRNA probe to visualize nitrogen-fixing cyanobacteria within ectodermal cells of another Caribbean coral (*Montastraea cavernosa*). Ainsworth et al. (2006) further optimized the method by using spectral fluorescence imaging, which allows the selection of fluorescent emissions that do not overlap with the autofluorescence spectrum of the coral. While high levels of autofluorescence derived from the Symbiodiniaceae and the coral tissues and non-specific binding of probes continue to be an issue (Wada et al., 2016), the former can be overcome by the use of fluorescence lifetime imaging microscopy (Deore et al., 2022). The power of FISH combined with three-dimensional confocal laser scanning microscopy was recently demonstrated when it revealed that a taxonomically diverse population of bacteria occur within Symbiodiniaceae cells, which had been overlooked or were indiscernible in earlier FISH and TEM studies on Symbiodiniaceae-containing coral tissue sections (Maire et al., 2021).

To further decipher the functions of coral-associated microbes as well as their interactions with each other and with their host, gene products or metabolites can be co-localized with microorganisms. For instance, in situ hybridization of coral tissue sections with probes targeting mRNAs showed that certain coral genes were only expressed in gastrodermal cells harbouring Symbiodiniaceae (Traylor-Knowles et al., 2017). Further, comparison of gene expression patterns between Symbiodiniaceae-containing and non-symbiotic gastrodermal cells of the coral *Stylophora pistillata* isolated via fluorescence-activated cell sorting demonstrated that certain metabolic pathways are only expressed in symbiotic cells. These include the complete pathway to degrade galactose, a major carbohydrate resource transported from symbiont to host and the pathway for glutathione production that likely protects the host from oxidative stress caused by algal photosynthesis (Levy et al., 2021). Another method that has been employed in corals to visualize and quantify the incorporation and cellular exchanges of isotopically labelled metabolites in situ is nanoscale secondary-ion mass spectrometry (NanoSIMS). This technique has a spatial resolution of

FIGURE 1 Detailed view of the cellular and structural diversity of reef-building corals. The region displayed is a close up of the area in red on the full coral polyp (top panel). The coloured band on the left-hand size highlight the different cellular layers and compartments. From top to bottom: seawater (dark blue); coral mucus (light blue); ectodermis: the outermost tissue layer of corals (peach); mesoglea: acellular and gel-like layer (red); gastrodermis: the inner cell layer that is lining the gastrovascular cavity and harbours Symbiodiniaceae cells (orange); gastrovascular cavity: the central body cavity of corals where digestion takes place (yellow); calicodermis: aboral ectoderm responsible for calcification (pink); calcium carbonate skeleton (beige); endolithic band: composed of filamentous algae (Genus: *Ostreobium*) together with fungi and prokaryotes (green). In addition, the circled numbers identify specific cellular structures: (1) epidermal cilia; (2) mucus-associated prokaryotes; (3) cnidocytes: stinging cells embedded in the ectodermis; (4) mucocyte: cells secreting mucus; (5) cell-associated microbial aggregates; (6) granular cells: cells involved in immune defence; (7) Symbiodiniaceae: family of photosynthetic microalgae (Phylum: Dinoflagellata) that form symbiotic associations with reef-building corals. Scale bar: 50 μm. Illustration: Philippe Plateaux.

50 nm and can be combined with TEM to identify metabolite enrichments in organelles, providing an unmatched view of subcellular scale processes. NanoSIMS was first applied to corals by Clode et al. (2007) and has since been used to examine assimilation and translocation of carbon, nitrogen and sulphur compounds by the coral host and Symbiodiniaceae (e.g., Higuchi et al., 2021; Kopp et al., 2013; Pernice et al., 2012; Rädecker et al., 2021), or bacteria (Lema et al., 2016; Rädecker et al., 2022). Recently, NanoSIMS was used in conjunction with FISH, laser capture microdissection and metagenomics to characterize the identity, genomic potential and metabolism of coral-associated microbial aggregates at high spatial resolution (Wada et al., 2022).

Another way to untangle microbial interactions is to use live imaging approaches. For example, underwater microscopy can non-invasively image corals in situ at the microbial scale (Mullen et al., 2016). In addition, interactions between coral and microbes within individual polyps, small fragments or larvae have been imaged using a microfluidic platform called the 'coral-on-a-chip' (Shapiro et al., 2016; Van Treuren et al., 2019). Using this device, the pattern of infection of coral by the fluorescently tagged bacterial pathogen, *Vibrio coralliilyticus*, was visualized with fluorescence microscopy (Shapiro et al., 2016). A variation to this approach was the inoculation of coral fragments with *V. coralliilyticus* labelled with the stable isotope ^{15}N to examine the fate of the bacterial cells within coral tissue sections using NanoSIMS and TEM (Gibbin et al., 2018). *V. coralliilyticus*-infected coral fragments were also incubated with ^{13}C to determine how carbon fixation by the Symbiodiniaceae and the translocation of photosynthate to the coral host was impacted by pathogen infection (Gibbin et al., 2019). The use of microfluidics for studying coral-microbe interactions is very promising but these tools have so far mostly been applied to pathogenic interactions with *V. coralliilyticus* (Gavish et al., 2021). However, a new microfluidic platform called the PhenoChip, has recently been used to identify individual Symbiodiniaceae cells with different photophysiological responses and tolerance thresholds (Behrendt et al., 2020; Xiao et al., 2022).

THE FUTURE OF SPATIAL ANALYSES IN CORAL SYMBIOSIS

Recent methodological breakthroughs in other symbiotic systems are likely to be transferable to corals. These advances include spatial metabolomics pipelines enabling the deciphering of host-microbe chemical interactions at micrometre resolution (Geier et al., 2019), correlative imaging workflows that connect mass spectrometry imaging to anatomic microstructures in three dimensions (Franke et al., 2021; Geier et al., 2021), simultaneous use of multiple taxon-specific FISH probes in an autofluorescent host (Ramírez-Puebla et al., 2022), or the selective capture of specific microbial cells coupled with low-input omics techniques (Ellis et al., 2021; Schede et al., 2021). These new capabilities will undoubtedly help to resolve the spatial distribution of specific coral symbionts, together with the genes they express or the metabolites they produce, contributing to illuminate their functional roles. Understanding which microbial functions underpin coral health is critical to the future of coral reefs. Climate change-driven increases in the frequency, severity and duration of summer heatwaves are causing an accelerating decline of coral reefs worldwide. The functional insights we will obtain by studying coral-associated microbes at the appropriate scale may drive novel microbiome manipulation strategies that, in addition to drastic reductions in our CO_2 emissions, will be required to safeguard future reefs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Madeleine J. H. van Oppen https://orcid.org/0000-0003-4607-0744

REFERENCES

Ainsworth, T.D., Fine, M., Blackall, L.L. & Hoegh-Guldberg, O. (2006) Fluorescence *in situ* hybridization and spectral imaging of coral-associated bacterial communities. *Applied and Environmental Microbiology*, 72, 3016–3020.

Apprill, A., Weber, L.G. & Santoro, A.E. (2016) Distinguishing between microbial habitats unravels ecological complexity in coral microbiomes. *mSystems*, 1, e00143-16.

Behrendt, L., Salek, M.M., Trampe, E.L., Fernandez, V.I., Lee, K.S., Kühl, M. et al. (2020) PhenoChip: A single-cell phenomic platform for high-throughput photophysiological analyses of microalgae. *Science Advances*, 6, eabb2754.

Bythell, J.C., Barer, M.R., Cooney, R.P., Guest, J.R., O'Donnell, A.G., Pantos, O. et al. (2002) Histopathological methods for the investigation of microbial communities associated with disease lesions in reef corals. *Letters in Applied Microbiology*, 34, 359–364.

Ceh, J., Kilburn, M.R., Cliff, J.B., Raina, J.B., van Keulen, M. & Bourne, D.G. (2013) Nutrient cycling in early coral life stages: *Pocillopora damicornis* larvae provide their algal symbiont (*Symbiodinium*) with nitrogen acquired from bacterial associates. *Ecology and Evolution*, 3, 2393–2400.

Clode, P.L., Stern, R.A. & Marshall, A.T. (2007) Subcellular imaging of isotopically labeled carbon compounds in a biological sample by ion microprobe (NanoSIMS). *Microscopy Research and Techniques*, 70, 220–229.

Deore, P., Wanigasuriya, I., Tsang Min Ching, S.J., Brumley, D.R., van Oppen, M.J.H., Blackall, L.L. et al. (2022) Fluorescence lifetime imaging microscopy (FLIM): a non-traditional approach to study host-microbial symbioses. *Microbiology Australia*, 43, 22–27.

Ellis, P., Moore, L., Sanders, M.A., Butler, T.M., Brunner, S.F., Lee-Six, H. et al. (2021) Reliable detection of somatic mutations in solid tissues by laser-capture microdissection and low-input DNA sequencing. *Nature Protocols*, 16, 841–871.

Franke, M., Geier, B., Hammel, J.U., Dubilier, N. & Leisch, N. (2021) Coming together - symbiont acquisition and early development in deep-sea bathymodioline mussels. *Proceedings of the Royal Society B: Biological Sciences*, 288, 20211044.

Gavish, A.R., Shapiro, O.H., Kramarsky-Winter, E. & Vardi, A. (2021) Microscale tracking of coral-vibrio interactions. *ISME Communications*, 1, 18.

Geier, B.K., Sogin, E., Michellod, D., Janda, M., Kompauer, M., Spengler, B. et al. (2019) Spatial metabolomics of *in situ*, host-microbe interactions. *Nature Microbiology*, 5, 498–510.

Geier, B., Oetjen, J., Ruthensteiner, B., Polikarpov, M., Gruber-Vodicka, H.R. & Liebeke, M. (2021) Connecting structure and function from organisms to molecules in small-animal symbioses through chemo-histo-tomography. *Proceedings of the National Academy of Sciences*, 118, e2023773118.

Gibbin, E., Gavish, A., Domart-Coulon, I., Kramarsky-Winter, E., Shapiro, O., Meibom, A. et al. (2018) Using NanoSIMS coupled with microfluidics to visualize the early stages of coral infection by *Vibrio coralliilyticus*. *BMC Microbiology*, 18, 39.

Gibbin, E., Gavish, A., Krueger, T., Kramarsky-Winter, E., Shapiro, O., Guiet, R. et al. (2019) *Vibrio coralliilyticus* infection triggers a behavioural response and perturbs nutritional exchange and tissue integrity in a symbiotic coral. *The ISME Journal*, 13, 989–1003.

Higuchi, T., Tanaka, K., Shirai, K., Yuyama, I., Mezaki, T., Takahata, N. et al. (2021) Sulfur assimilation in corals with aposymbiotic and symbiotic zooxanthellae. *Environmental Microbiology Reports*, 13, 98–103.

Hughes, D.J., Raina, J.-B., Nielsen, D.A., Suggett, D.J. & Kühl, M. (2022) Disentangling compartment functions in sessile marine invertebrates. *Trends in Ecology & Evolution*, 37, 740–748.

Kimes, N.E., Van Nostrand, J.D., Weil, E., Zhou, J. & Morris, P.J. (2010) Microbial functional structure of *Montastraea faveolata*, an important Caribbean reef-building coral, differs between healthy and yellow-band diseased colonies. *Environmental Microbiology*, 12, 541–556.

Kopp, C., Pernice, M., Domart-Coulon, I., Djediat, C., Spangenberg, J.E., Alexander, D.T.L. et al. (2013) Highly dynamic cellular-level response of symbiotic coral to a sudden increase in environmental nitrogen. *mBio*, 4, e00052-13.

Kuek, F.W.I., Motti, C.A., Zhang, J., Cooke, I.R., Todd, J.D., Miller, D. J. et al. (2022) DMSP production by coral-associated bacteria. *Frontiers in Marine Science*, 9, 869574.

Lema, K., Clode, P., Kilburn, M., Thornton, R., Willis, B.L. & Bourne, D.G. (2016) Imaging the uptake of nitrogen-fixing bacteria into larvae of the coral *Acropora millepora*. *The ISME Journal*, 10, 1804–1808.

Lesser, M.P., Mazel, C.H., Gorbunov, M.Y. & Falkowski, P.G. (2004) Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. *Science*, 305, 997–1000.

Levy, S., Elek, A., Grau-Bové, X., Menéndez-Bravo, S., Iglesias, M., Tanay, A. et al. (2021) A stony coral cell atlas illuminates the molecular and cellular basis of coral symbiosis, calcification, and immunity. *Cell*, 184, 2973–2987.

Maire, J., Girvan, S.K., Barkla, S.E., Perez-Gonzalez, A., Suggett, D. J., Blackall, L.L. et al. (2021) Intracellular bacteria are common and taxonomically diverse in cultured and in hospite algal endosymbionts of coral reefs. *The ISME Journal*, 15, 2028–2042.

Marcelino, V.R., van Oppen, M.J.H. & Verbruggen, H. (2018) Highly structured prokaryote communities exist within the skeleton of coral colonies. *The ISME Journal*, 12, 300–303.

Mullen, A.D., Treibitz, T., Roberts, P.L.D., Kelly, E.L.A., Horwitz, R., Smith, J.E. et al. (2016) Underwater microscopy for *in situ* studies of benthic ecosystems. *Nature Communications*, 7, 12093.

Neave, M.J., Apprill, A., Ferrier-Pages, C. & Voolstra, C.R. (2016) Diversity and function of prevalent symbiotic marine bacteria in the genus *Endozoicomonas*. *Applied Microbiology and Biotechnology*, 100, 8315–8324.

Pernice, M., Meibom, A., Van Den Heuvel, A., Kopp, C., Domart-Coulon, I., Hoegh-Guldberg, O. et al. (2012) A single-cell view of ammonium assimilation in coral-dinoflagellate symbiosis. *The ISME Journal*, 6, 1314–1324.

Pollock, F.J., McMinds, R., Smith, S., Bourne, D.G., Willis, B.L., Medina, M. et al. (2018) Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nature Communications*, 9, 4921.

Rädecker, N., Pogoreutz, C., Gegner, H.M., Cárdenas, A., Roth, F., Bougoure, J. et al. (2021) Heat stress destabilizes symbiotic nutrient cycling in corals. *Proceedings of the National Academy of Sciences*, 118, e2022653118.

Rädecker, N., Pogoreutz, C., Gegner, H.M., Cárdenas, A., Perna, G., Geißler, L. et al. (2022) Heat stress reduces the contribution of diazotrophs to coral holobiont nitrogen cycling. *The ISME Journal*, 16, 1110–1118.

Ramírez-Puebla, S.T., Weigel, B.L., Jack, L., Schlundt, C., Pfister, C. A. & Mark Welch, J.L. (2022) Spatial organization of the kelp microbiome at micron scales. *Microbiome*, 10, 52.

Ritchie, K.B. (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Marine Ecology Progress Series*, 322, 1–14.

Schede, H.H., Schneider, C.G., Stergiadou, J., Borm, L.E., Ranjak, A., Yamawaki, T.M. et al. (2021) Spatial tissue profiling by imaging-free molecular tomography. *Nature Biotechnology*, 39, 968–977.

Shapiro, O.H., Kramarsky-Winter, E., Gavish, A.R., Stocker, R. & Vardi, A. (2016) A coral-on-a-chip microfluidic platform enabling live-imaging microscopy of reef-building corals. *Nature Communications*, 7, 10860.

Traylor-Knowles, N., Rose, N.H. & Palumbi, S.R. (2017) The cell specificity of gene expression in the response to heat stress in corals. *The Journal of Experimental Biology*, 220, 1837–1845.

Van Treuren, W., Brower, K.K., Labanieh, L., Hunt, D., Lensch, S., Cruz, B. et al. (2019) Live imaging of Aiptasia larvae, a model system for coral and anemone bleaching, using a simple microfluidic device. *Scientific Reports*, 9, 9275.

Wada, N., Pollock, F.J., Willis, B.L., Ainsworth, T., Mano, N. & Bourne, D.G. (2016) *In situ* visualization of bacterial populations in coral tissues: pitfalls and solutions. *PeerJ*, 4, e2424.

Wada, N., Hsu, M.T., Tandon, K., Hsiao, S.S.-Y., Chen, H.-J., Chen, Y.-H. et al. (2022) High-resolution spatial and genomic characterization of coral-associated microbial aggregates (CAMAs) in the coral *Stylophora pistillata*. *Science Advances*, 8, eabo2431.

Welch, J.L.M., Rossetti, B.J., Rieken, C.W., Dewhirst, F.E. & Borisy, G.G. (2016) Biogeography of a human oral microbiome at the micron scale. *Proceedings of the National Academy of Sciences*, 113, E791–E800.

Xiao, L., Johansson, S., Rughöft, S., Burki, F., Sandin, M.M., Tenje, M. et al. (2022) Photophysiological response of Symbiodiniaceae single cells to temperature stress. *The ISME Journal*, 16, 2060–2064.

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