Coral Reefs of the World 20

Raquel S. Peixoto
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Editors

Coral Reef Microbiome

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The book strives to foster innovative, science-based decision-making to significantly advance efforts in coral conservation and restoration. We introduce the term "Assisted Restoration" (AR) as the sum of approaches required to deliver effective ecological restoration. AR signifies a multidisciplinary strategy with which innovative tools are integrated into routine practices of coral restoration to build resilience and increase their survival in a changing world. As such, it aligns with and expands on the notion of Coral Assisted Evolution by Madeleine van Oppen, James Oliver, Hollie Putnam, and Ruth Gates into a broader picture, considering and combining any intervention that may build resilience. We therefore dedicate this book to the inspiring Ruth Gates, whose vision, support, and enthusiasm continue to guide us. You are deeply missed.

Preface

Microorganisms, as catalysts of all biogeochemical cycles on our planet, are the very origin and essence of life on Earth—an invisible yet formidable force that sustains all living organisms. The health of individual organisms and their ecosystems depend critically on functional microbiomes that support essential processes such as nutrient cycling, pathogen control, detoxification, and resilience. However, like their macroorganismal counterparts, these beneficial commensal microbes are vulnerable to environmental changes, and their decline often accelerates ecosystem degradation.

Anthropogenic impacts have profoundly changed and often degraded most ecosystems and their microbiomes, and coral reefs are no exception. Stony corals, the foundation species of these vibrant ecosystems, are among the most threatened marine organisms. Shifts in coral microbiomes toward dysbiotic (i.e., harmful) assemblages are increasingly recognized as both a cause and consequence of coral mortality. To counter this, active intervention strategies are being developed and applied to restore and rehabilitate degraded microbiomes with the goal of preserving and reestablishing beneficial microbial communities that underpin the health of their respective host organisms and ecosystems.

The success of these approaches relies on our understanding of the distribution, ecological roles, and interactions between corals and their associated microbiomes, how environmental factors impact them, and their potential to amplify or mitigate anthropogenic impact. This book explores these aspects, how they shape coral holobiont assemblages, and how they provide a gateway to active intervention. Furthermore, the book offers a practical, customized, and adaptable roadmap for stakeholders to incorporate most recent insights into a broader One Health framework and ecosystem perspective.

The book strives to foster innovative, science-based decision-making to significantly advance efforts in coral conservation and restoration. We introduce the term "Assisted Restoration" (AR) as the sum of approaches required to deliver effective ecological restoration. AR signifies a multidisciplinary strategy with which innovative tools are integrated into routine practices of coral restoration to build resilience and increase their survival in a changing world. As such, it aligns with and expands on the notion of Coral Assisted Evolution by Madeleine van Oppen, James Oliver, Hollie Putnam, and Ruth Gates into a broader picture, considering and combining any intervention that may build resilience. We therefore dedicate this book to the inspiring Ruth Gates, whose vision, support, and enthusiasm continue to guide us. You are deeply missed.

Prof. Peixoto is a microbial ecologist investigating the diversity, ecological role, and biotechnological potential of microorganisms associated with marine organisms. Her pioneering work has outlined the protocols and proved the concept that the use of microbial therapies and coral probiotics is possible and can increase the host's resilience and resistance against environmental threats. She is currently the President of the International Society for Microbial Ecology (ISME) and focused on highlighting the importance of microbes to promote organism and ecosystem health and fight climate change and their impacts, as well as the key role of microbiologists in planetary health discussions.

Prof. Voolstra holds the Professorial Chair of Genetics of Adaptation in Aquatic Systems at the University of Konstanz. His most recent research particularly advanced knowledge of how the microbiome contributes to coral acclimation/adaptation and the delineation of standardized analytical methods (SymPortal, CBASS short-term acute thermal stress assays, Coracle, etc.) to fasten the development of approaches to mitigate climate change. He is an advocate of open science, open access, and free data sharing. He is currently the President of the International Coral Reef Society (ICRS) and advocates for the urgency of developing and applying science-based solutions to coral reefs following a rigorous, evidence-based decision framework.

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Part I

Introduction

Introduction to *Coral Reef Microbiome*

1

Raquel S. Peixoto and Christian R. Voolstra

Abstract

Coral reefs are important marine ecosystems, supporting immense biodiversity and providing significant ecological and economic benefits. Corals and the reefs they build face unprecedented decline from local and global anthropogenic pressure. Central to the health and resilience of coral reefs is the coral microbiome, a complex community of microorganisms that inhabit coral tissues, mucus, skeleton, and the surrounding environment. Microbial communities play a critical role in maintaining coral health, mediating nutrient cycling, and enhancing stress tolerance. Besides host-associated microbes, environmental microorganisms shape the reef ecosystem and its functions. The importance of host- and ecosystem-associated microbiomes, our ability to alter their structure and function, and the ongoing decline of coral reefs led to the notion of 'microbiome stewardship', i.e. the management of the microbiome to restore/rehabilitate organisms and ecosystem function, increase resilience, and counter biodiversity loss. This book starts with dedicated chapters to detail the most important microbial associates of corals. It then outlines the emergent coral holobiont and the underlying structuring forces to subsequently transition over to ecosystem-scale microbiology and health of coral reefs. After that, the book explores how microbial processes contribute to coral disease, highlights microbial therapies for restoring coral health, and provides a roadmap for their implementation. The book concludes with a discussion on coral microbiome biotechnology and a forward-looking perspective on enhancing coral reef restoration through adaptive interventions and data science under a One-Health concept. This chapter provides an introduction to the book, briefly highlighting the various chapters.

Keywords

Coral Reef · Climate Change · Microbiome · Bacteria · Resilience · Health · Environmental adaptation

Coral reefs are not only spectacular marine ecosystems, but also hotspots of biodiversity that serve as crucial pillars of marine life (Knowlton et al. 2021). They provide habitats for thousands of marine species and are integral to the livelihoods of millions of people worldwide (Costanza et al. 1997, 2014). At the core of corals and coral reefs, complex microbiomes composed of representatives from all domains of life play essential roles in maintaining their health, resilience, and the functions of these ecosystems, besides contributing to ecosystem services (Voolstra et al. 2024). Healthy organisms and ecosystems therefore rely on healthy microbiomes, yet these beneficial or commensal microbes are often sensitive to shifts in environmental conditions.

This book, "Coral Reef Microbiome", aims to provide a comprehensive description of the coral- and reef-associated microbiomes, their interactions, their contribution to organismal and ecosystem health, and the prospect of microbiome management as a target for restoration/rehabilitation. The book concludes by highlighting the biotechnological potential of the coral reef microbiome and how standardization, automation, and artificial intelligence will impact coral reef research and conservation.

The first chapters introduce the most prevalent microbiome members of stony corals and reef ecosystems. Chapter 2 opens our exploration by focusing on the family Symbiodiniaceae (LaJeunesse et al. 2018; Davies et al. 2023; Parkinson et al. 2022), the primary photo endosymbionts of corals, which are vital for their survival (Muscatine 1990).

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These algae provide upwards of 90% of the corals' energetic requirements through the translocation of their photosynthates, contributing significantly to coral health, growth, and calcification (Muscatine 1990). Recognizing, describing, and appreciating the tremendous diversity of these microalgal dinoflagellates is a critical component of our ability to forecast how coral reefs will be able to adapt to changing environmental conditions. Chapter 3 expands our perspective to include Bacteria and Archaea, examining their roles across different reef habitats—from the water column to sediments to host-associated—and their critical functions in nutrient cycling and maintaining coral and reef health (Weber et al. 2019; Apprill et al. 2023; Voolstra et al. 2024; Bourne et al. 2016). Chapter 4 explores the coral skeleton's hidden residents, the endolithic algae, particularly the genus *Ostreobium* (Pushpakumara et al. 2023; Marcelino et al. 2018; Pernice et al. 2020; Cárdenas et al. 2022). These overlooked algae play an important role in the biology, ecology, and resilience of corals, demonstrating the complexity of life within the coral holobiont. Chapter 5 discusses viruses and their dominant role in shaping the biology of ecosystems and organisms. Viruses affect everything from nutrient cycles to disease dynamics in coral reefs, influencing microbial populations and coral health (Silveira et al. 2023; Voolstra et al. 2024; Silveira and Rohwer 2016). We conclude the different microbial entities with Chapter 6, which explores the world of fungi and fungi-like organisms within corals and reef ecosystems, examining their diversity, functional roles, and potential contributions to coral health and reef resilience, highlighting the need for further research to unlock the mysteries of these enigmatic microeukaryotes (Roik et al. 2022).

In the chapters that follow, we look at the emergent complexities that arise from microbial-microbial and host-microbial interactions. Chapter 7 describes how the diverse microbial representatives individually described in Chapters 1–6 form complex interactions to comprise the coral metaorganism or holobiont (i.e., the coral host and the associated microbiomes and their interactions) (Knowlton and Rohwer 2003; Rohwer et al. 2002; Bosch and McFall-Ngai 2011). Chapter 8 then explores how microbiome composition varies according to coral species, environmental condition, and host genotype (Voolstra and Ziegler 2020; Neave et al. 2017; Ziegler et al. 2019), in addition to discussing the roles of resident, transient, and core microbial communities for coral health and environmental adaptation (Hernandez-Agreda et al. 2017; Bourne et al. 2016; Jaspers et al. 2019).

We then move beyond the holobiont to explore the interactions between corals and their environment. Chapter 9 details the critical roles of microorganisms and coral microbiomes in nutrient cycling within coral reefs, detailing how corals thrive in nutrient-poor waters while paying tribute to the importance of coral heterotrophy (Voolstra et al. 2024; Raina et al. 2009; Bourne et al. 2016; Ceh et al. 2013). Chapter 10 provides a comprehensive account of reef-associated microbial diversity, the underlying structuring forces, and their contribution to reef function to provide a view of ecosystem-scale microbiology (Bourne et al. 2016; Thurber et al. 2017). This section ends with Chapter 11, highlighting the importance of microbial and viral communities in contributing to reef health at large through the interacting processes of microbialization and viralization (Haas et al. 2016; Silveira et al. 2023). Both processes describe the dramatic shifts in microbial/viral communities that coral reefs can experience when exposed to environmental stressors such as pollution, overfishing, and climate change contributing to coral disease, reduced resilience, and overall ecosystem decline. Both phenomena underscore the critical role of microbial balance in maintaining the health and stability of coral reef ecosystems. Chapters 1–11 conclude the description of the microbiology of corals and reef ecosystems.

In the following chapters, we then turn our attention to host-microbial interactions as a culprit when interactions go wrong and as a remedy to apply in the form of microbial-based therapies. Chapter 12 examines the role of the microbiome in coral diseases, bleaching, and dysbiosis, and discusses current research on coral pathogens and the complex dynamics leading to coral decline (Sweet and Bulling 2017; Paul et al. 2019; Ushijima et al. 2020; Meyer et al. 2017). Conversely, Chapter 13 explores ways to employ microbial-based therapies to rehabilitate dysbiotic coral holobionts (Raquel S. Peixoto et al. 2017; C. R. Voolstra et al. 2021; R. S. Peixoto et al. 2019; Garcias-Bonet et al. 2023) following the concept of microbiome stewardship, i.e. the management of microbiomes to restore organismal and ecosystem health and biodiversity (Peixoto et al. 2022). Chapter 14 introduces ethical, legal, and ecological considerations (Suggett et al. 2023) in deploying microbial-based interventions for coral conservation and restoration. It proposes a rights-based framework (Camp et al. 2024) to guide decisions on the use of such active interventions, emphasizing the high risk of inaction (Peixoto et al. 2022) and a decision framework for the implementation of ethical considerations.

We conclude the book by providing a forward-looking perspective on biotechnology and how emergent technologies will impact coral conservation/restoration. Chapter 15 explores the biotechnological potential of the coral microbiome, discussing applications in medicine and industry, which represents a potential key contribution of coral-associated microbes to our society that may support conservation and restoration efforts (Modolon et al. 2020; Sweet et al. 2021; Keller-Costa et al. 2022). Finally, Chapter 16 synthesizes the insights gathered throughout the book, emphasizing the importance of multidisciplinary approaches to conservation and restoration and highlighting the interconnectedness of microbial actions with the health of coral reefs (Peixoto et al.

2024a, b). Such consideration leads to the novel concept of "assisted restoration", i.e. the combination of cutting-edge coral restoration technologies supported by, for instance, microbial-based therapies, which can surpass current restoration outcomes by improving stress tolerance and extending the resilience of restored biomass. In addition, this chapter details how automation, standardization, and artificial intelligence will impact the coral reef conservation and restoration landscape (Voolstra et al. 2025).

We hope you enjoy the comprehensive exploration of the fascinating world of coral microbiomes and, by the end of your reading, have further extended your passion for the topic with us. Throughout this book, we aim to offer a comprehensive, insightful, and accessible perspective on the coral microbiome, offering both foundational knowledge and the latest research findings to students, scientists, and practitioners interested in marine biology and the preservation of one of Earth's most vital and mesmerizing ecosystems: coral reefs.

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Part II

Microbial Compartments in the Reef and Coral Holobiont

Symbiodiniaceae

2

John Everett Parkinson, Raquel S. Peixoto,
and Christian R. Voolstra

Abstract

The primary photosymbionts of tropical reef-building corals belong to the microalgal family Symbiodiniaceae. These eukaryotic dinoflagellates, commonly known as 'zooxanthellae,' form intracellular associations with cnidarian hosts and represent a key nutritional component of the coral microbiome. The cnidarian-dinoflagellate mutualism fuels coral skeletal growth, generating the three-dimensional habitat that supports the immense diversity of reef-associated organisms and builds the foundation of reef ecosystems to provide ecological goods and services. To predict how coral reefs will respond in a warming world, it is vital to comprehensively describe the extant biodiversity underlying Symbiodiniaceae. Such a catalog serves as a foundation to understand the adaptive capacity of the algal symbionts, the coral hosts, and the combined holobiont. This overview chapter is targeted at microbiome researchers who may be new to the coral reef community and Symbiodiniaceae world (welcome!). It briefly covers several important aspects of symbiodiniacean biology: their energetic contribution to reefs, their astounding diversity, their role in coral bleaching, their molecular interactions with hosts, their evolutionary history, and their own microbial associations. It concludes with advice for interpreting past literature while developing new research.

Keywords

Dinoflagellates · Eukaryotic microbes · Photosymbionts · *Symbiodinium* · Zooxanthellae

2.1 The Engine of the Reef

The mutualism between cnidarian hosts and symbiodiniacean symbionts forms the energetic foundation of coral reef ecosystems through photobiological nutritional interactions (reviewed by Muscatine 1990; Roth 2014; Pogoreutz et al. 2020; Rädecker et al. 2023). Coral hosts benefit from the photosynthetically-fixed carbon (sugars) the algae provide, but they can also acquire nutrients through small prey capture via stinging nematocysts and tentacles (reviewed by Houlbrèque and Ferrier-Pagès 2009). There are many successful, nonsymbiotic corals in colder, more nutrient-rich environments, but their colonies tend not to form large skeletal structures and they don't support large reefs (Vuleta et al. 2024). Conversely, many reef-building corals in tropical environments use the autotrophic products of their microalgal symbionts to supplement heterotrophic energy intake (while heterotrophy may compensate for autotrophic energy loss when in a bleached state; Grottoli et al. 2006). The energy the Symbiodiniaceae harness from sunlight and transfer to their hosts is sufficient to allow for relatively rapid skeletal growth: a process eventually known as light-enhanced calcification (Kawaguti and Sakamoto 1948; Goreau 1959). For decades it was believed that glycerol is the primary sugar transferred to the host, but recent metabolomic evidence points to glucose (Burriesci et al. 2012). It is clear that without this added source of metabolic fuel, corals are incapable of calcifying at rates that generate reef habitat, and without this habitat, the unique and vibrant reef ecosystem cannot take shape.

Reef ecosystems are critical to the planet for a number of reasons. The most important derives from the immense

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biodiversity associated with tropical reefs, which occupy a small proportion of Earth's surface and yet account for relatively large proportions of the ocean's phyletic diversity (Paulay 1997; Spalding et al. 2001). About 30% of described marine multicellular species depend on coral reefs (Fisher et al. 2015). Reefs provide the three-dimensional structure that facilitates multiple biological processes. Organisms use reef habitat as nurseries, hunting grounds, and shelter (Hixon and Beets 1993; Nagelkerken et al. 2000). Reefs attract animals that otherwise would not congregate and interact. In essence, corals build reefs that transform oceanic deserts into the rainforests of the sea. Coral reefs also provide many critical ecological goods and services that support humans (reviewed by Moberg and Folke 1999). Some of the most important economically include fisheries, shoreline protection, tourism, biopharmaceuticals, and other biotechnological products (see Chapter 15). The monetary value extracted from reefs annually is estimated in the billions to trillions of US dollars (Conservation International 2008; de Groot et al. 2012; Hoegh-Guldberg 2015), and none of it would be possible without coral photosymbionts. From this perspective, Symbiodiniaceae represent one of the most valuable groups of microbes on the planet.

2.2 Symbiodiniaceae Diversity

Most reef-building coral species form obligate associations with microalgae in the family Symbiodiniaceae (LaJeunesse et al. 2018). Unlike most other microbial components of the coral holobiont, symbiodiniaceans are eukaryotic, just like their hosts. The algal cells reside within host endodermal cells inside a vacuolar membrane called the symbiosome (Wakefield et al. 2000), so the association is considered endosymbiotic. Phylogenetically, dinoflagellates are placed in the same super-group as alveolates and share common ancestry with parasitic apicomplexans. A wide diversity of marine invertebrates can form symbioses with Symbiodiniaceae (reviewed by Trench 1993). Beyond reef-building corals, potential hosts include other cnidarians (sea anemones, sea fans, jellies, zoanthids) as well as sea slugs, giant clams, flatworms, and sponges. Unicellular hosts include ciliates and forams. In addition, there are some exclusively free-living Symbiodiniaceae species that can be found in coral reefs (Takabayashi et al. 2012); their ecological relevance is not yet fully understood (Fujise et al. 2020).

Species recognition is the critical prerequisite to investigating biodiversity, ecology, evolution, and conservation, but it can be quite difficult to morphologically resolve different species of Symbiodiniaceae (Trench and Blank 1987). As dinoflagellates, symbiodiniaceans possess two flagella that they lose *in hospite*. Other noteworthy morphological features include armored plates (theca), an anchoring body (pilus), a special organelle for carbon concentration (pyrenoid), and a network of highly reticulated chloroplasts (LaJeunesse et al. 2012b, 2018). However, these traits are rarely diagnostic, and even cell size is an unreliable metric for taxonomic classification as there can be large variation within a species and overlap between species (LaJeunesse et al. 2018). As a result, DNA sequencing has become the primary means for identifying different algal lineages (reviewed by Davies et al. 2023). Historically, the most popular marker has been the hypervariable Internal Transcribed Spacer 2 (ITS2) rDNA region, a non-coding region that separates the 18S and 28S rRNA genes, which gave rise to an alphanumeric designation system for different phylotypes, referred to as 'Clades' and 'subclades' (e.g., A1, B7, D1a, etc.) to acknowledge the tremendous extant genetic diversity (LaJeunesse 2001). Though these subcladal phylotypes can approximate species-level designations, the ITS2 marker has limitations. Because each symbiodiniacean genome contains multiple ITS2 copies, there is both inter- and intra-specific sequence variation to account for (Thornhill et al. 2007; Arif et al. 2014). Additionally, the marker evolves at different rates in different lineages, and therefore its resolving power varies (Pochon et al. 2014). For the purpose of describing new species and assigning binomials, ITS2 alone is insufficient; it is necessary to sequence multiple molecular markers and incorporate ecological, physiological, and morphological data whenever possible (LaJeunesse et al. 2012a). As a result, the number of validly described Symbiodiniaceae species (44 at present; Appendix 1) represents just a fraction of the inferred diversity based on ITS2 phylotypes (in the hundreds; reviewed by LaJeunesse et al. 2018). A current effort focuses on consolidating multi-marker species level descriptions with next-generation sequencing ITS2 type profiles (derived from SymPortal; Hume et al. 2019) to map the extant molecular diversity onto genotype-, population-, and species-level diversity.

The great molecular diversity of Symbiodiniaceae is also associated with great ecological and physiological diversity (Table 2.1; reviewed by Nitschke et al. 2022). With respect to ecology, some lineages are strictly free-living and therefore considered nonsymbiotic (Jeong et al. 2014), while others appear to be obligate symbionts (Krueger and Gates 2012), and still others can thrive in either state (LaJeunesse et al. 2014). Some are host-specific (Thornhill et al. 2014), while others are generalists that can associate with a range of hosts (Butler et al. 2023). Some exhibit parasitic tendencies (LaJeunesse et al. 2015), and some are capable of heterotrophy (Jeong et al. 2012). Physiologically, Symbiodiniaceae species vary in terms of tolerance to heat (Díaz-Almeyda et al. 2017) and cold (Thornhill et al. 2008), as well as optimum levels of light (Robison and Warner 2006), salinity (Rogers and Davis 2006; Ochsenkühn et al. 2017), nutrients (Rodriguez-Roman and Iglesias-Prieto 2005), and pH (Brading et al. 2011). Even

Table 2.1 The ecological diversity of a subset of Symbiodiniaceae genera and species

Example Genus	Example Species	Notable Details
Breviolum	*B. dendrogyrum*	Host-specialist. Only associates with the Caribbean coral *Dendrogyra cylindrus*.
	B. endomadracis	Endosymbiont of corals in the genus *Madracis* and potentially Caribbean octocorals.
	B. minutum	Symbiont of tropical sea anemones, among others. First published dinoflagellate genome.
	B. psygmophilum	Cold-tolerant Atlantic symbiont of stony corals, octocorals, and sea anemones.
Cladocopium	*C. infistulum*	Giant clam symbiont (extracellular). Heat-tolerant compared to *S. tridacnidorum*.
	C. pacificum	Vertically-transmitted symbiont specific to Indo-Pacific pocilloporid corals.
	C. proliferum	Host-generalist common in corals from the central to northern Great Barrier Reef.
	C. thermophilum	Extremely heat-tolerant coral symbiont from the world's hottest sea: the Persian/Arabian Gulf.
Durusdinium	*D. boreum*	Symbiont of the coral *Oulastrea crispata*. Adapted to sub-tropical/temperate waters.
	D. eurythalpos	Symbiont of the coral *Oulastrea crispata*. Adapted to tropical/sub-tropical waters.
	D. glynnii	Stress-tolerant symbiont of Eastern Tropical/Sub-Tropical Pacific pocilloporid corals.
	D. trenchii	Heat-tolerant host-generalist. Widespread and likely invasive in the Caribbean.
Symbiodinium	*S. microadriaticum*	Caribbean symbiont of jellies. First (invalidly) described Symbiodiniaceae species.
	S. necroappetens	Opportunistic, likely necrotrophic species that proliferates in bleached and diseased corals.
	S. pilosum	Relatively large, free-living, non-symbiotic species from the Caribbean with notable pili.
	S. tridacnidorum	Giant clam symbiont (extracellular). Heat-sensitive compared to *C. infistulum*.

Although it is impossible to portray the characteristics of all the named and unnamed Symbiodiniaceae in one table, four genera that commonly associate with cnidarian hosts are featured, each represented by four species. The brief descriptions of species-level traits are meant to emphasize variation within and among genera. For a full list of taxonomically described genera and species, see Appendix 1

within a species, physiological diversity can be extreme and impact host-symbiont interactions (Hawkins et al. 2016; Parkinson et al. 2022). When reared under identical conditions, variation in growth rate and photochemical efficiency among conspecific strains can exceed average differences between species (Parkinson and Baums 2014; Díaz-Almeyda et al. 2017). Such great physiological diversity, coupled with large population sizes and rapid generation times, indicates a large adaptive capacity within Symbiodiniaceae populations (van Oppen and Medina 2020). Indeed, experimental evolution via heat ratcheting has successfully driven thermal tolerance phenotypes of initially clonal strains to diverge by several degrees Celsius in just 2.5 years (~80 generations; Chakravarti et al. 2017). This adaptive capacity is critical in the context of coral bleaching (see below).

2.3 Coral Bleaching

The coral-algal mutualism is delicate. If conditions become stressful, it can fail, changing from mutualism to competition (Rädecker et al. 2021, 2023). Nutrients may no longer be exchanged optimally, and the partners no longer tolerate each other's presence. The algal cells exit the colony in a process termed coral bleaching (Fitt et al. 2001). If enough algae or their pigments are lost, affected colonies appear pale or completely white as the calcium carbonate skeleton beneath the translucent coral tissue becomes visible. The thermal sensitivity of a coral holobiont is determined by the host, the algal symbiont, the wider microbial community, and their interactions (reviewed by Voolstra et al. 2021). It is not necessarily helpful to think of either partner as being in “control” (e.g., the coral kicks out the algae or the algae escape the coral; LaJeunesse 2020). Bleaching is the combined result of a breakdown in stabilizing molecular cross-talk and metabolic compatibility between partners (Suggett and Smith 2020). Once interactions become dysbiotic, host innate immune responses are turned on. Multiple mechanisms of dissociation may activate, such as exocytosis of algal cells from the host cells, symbiophagy, host autophagy, and host apoptosis (reviewed by Weis 2008; Helgoe et al. 2024). Some of these mechanisms are still active even after environmental conditions have returned to normal (Santoro et al. 2021). Coral bleaching is a generalized stress response that can be triggered through environmental changes (e.g., hot or cold temperatures, salinity fluctuations, darkness) or biotic changes (e.g., host disease, bacterial pathogens, etc.; reviewed by Helgoe et al. 2024). If the stress subsides, colonies can recover from bleaching through regrowth of remnant algal populations within their tissues, or uptake of algae from the environment (with limitations, further discussed below; reviewed by Baker 2003).

Despite decades of research, the exact molecular mechanisms leading to bleaching are not completely understood. The long-established oxidative hypothesis for heat-induced bleaching posits that a combination of high temperature and high light damages the photoreactive centers in the symbiont’s chloroplasts, causing a build-up of reactive oxygen species (ROS) that leach into the host, drive cellular damage, and incite bleaching (Lesser 1996; Downs et al. 2002; Weis 2008). While many experiments support aspects of this sequence, there are some problems. For example, host redox responses are not necessarily coupled to symbiont photophysiology (Krueger et al. 2015) and ROS buildup is neither the direct (Nielsen et al. 2018; Dungan et al. 2022) nor the proximate (Schlotheuber et al. 2024) cause of bleaching. The more recent (and non-mutually exclusive) carbon limitation hypothesis has focused on stress-induced fluctuations in carbon and nitrogen exchange between rather unequal partners (Wooldridge 2009; Rädecker et al. 2015). Simply put, the coral host domesticates its algae to release sugars under nitrogen-limited conditions in return for CO_2 and a light-rich environment. This nutrient cycling symbiosis is self-perpetuating and self-regulated under ambient conditions but becomes unstable under stressful conditions (e.g. excess warming or eutrophication; Rädecker et al. 2021, 2023). Ultimately, just as there are many environmental triggers for bleaching, there are likely many molecular mechanisms that contribute to bleaching, and their importance and degree of overlap likely varies depending on the association and stressor (Helgoe et al. 2024).

Although coral bleaching is clearly a stress response indicative of a major dysbiosis, it has also been suggested that bleaching can serve as an adaptive mechanism–a way to respond to a changing environment by altering the composition of the colony's symbiont community (Buddemeier and Fautin 1993). For example, corals dominated by thermally-sensitive algal symbionts sometimes bleach under heat stress and recover with a greater proportion of thermally-tolerant symbionts, making them more capable of withstanding future heat stresses (Silverstein et al. 2015; Palacio-Castro et al. 2023). Arguing against the adaptive quality of these changes is the fact that such changes are often temporary, with the algal assemblage shifting back to the original composition over time in the absence of stress (Thornhill et al. 2006; LaJeunesse et al. 2010). However, if the stress persists, so can the new symbiont community (Silverstein et al. 2017; Manzello et al. 2018). These observations indicate that there are trade-offs involved with algal symbiont community shifts, at least in the Caribbean where the most quickly spreading heat-tolerant symbiont (*Durusdinium trenchii*) appears to be invasive (Pettay et al. 2015). Note, however, that *D. trenchii* shows no nutrient exchange trade-offs under heat stress in its original Indo-Pacific range with its typical host species (Kemp et al. 2023). In a study case from the Persian-Arabian Gulf, the warmest ocean basin where corals live, the environment is so extreme that otherwise rare, pre-adapted algal symbionts of the species *Cladocopium thermophilum* became selected and spread across the resident coral hosts (Hume et al. 2016). Factors that can shape which particular symbiodiniacean species dominates a colony pre- and post-bleaching include host and symbiont specificity (Gabay et al. 2018), priority effects (Gabay et al. 2019), opportunism (LaJeunesse et al. 2015), competition (McIlroy et al. 2019), optimal nutrient exchange (Matthews et al. 2017), degree of heat tolerance in a particular host background (DeSalvo et al. 2010; Cunning et al. 2015), and disturbance history (Claar et al. 2020), among others.

There are some peculiarities about coral bleaching that are important to keep in mind. First, the strict definition of bleaching refers only to a loss of algal cells or pigments, which can occur in the absence of stress (Fitt et al. 2001). A healthy coral expels millions of healthy algae every day (Stimson and Kinzie 1991), which likely prevents the symbiont population from exceeding the available space in the host (Jones and Yellowlees 1997), as well as syncing of cell division between host and algal symbionts based on nitrogen availability (Rädecker et al. 2021, 2023). Symbiont density also fluctuates seasonally (Stimson 1997). If this natural off-ramp didn't exist, an equilibrium between partners couldn't be maintained. Second, just because a colony is visibly bleached doesn't mean that there aren't still millions of Symbiodiniaceae cells remaining in its tissue (Siebeck et al. 2006). In fact, the symbiont cells within (and expelled from) bleached colonies may be healthy even though the coral host is suffering (Bhagooli and Hidaka 2004). Third, pale colonies are not necessarily stressed (Cruz et al. 2015), and colorful colonies are not necessarily healthy (Bonesso et al. 2017). Paleness can reliably indicate the health status of some species, but not others (Parkinson et al. 2016).

2.4 Molecular Interactions

The molecular interactions between corals and Symbiodiniaceae are poorly understood when compared to other symbiosis models, such as *Hydra-Chlorella* or legume-rhizobia associations. Nevertheless, progress has been made in clarifying aspects of host-symbiont recognition, the development of specificity, and the establishment and maintenance of healthy symbiont populations within coral tissues (reviewed by Davy et al. 2012). Most corals form highly specific symbioses with particular Symbiodiniaceae taxa (reviewed by Thornhill et al. 2017). Among unique genotypes of individual coral colonies, often just one (or a few) clonal lineage(s), i.e., genotypes, of one algal species represent(s) the numerically dominant member of the symbiont community (Baums et al. 2014; Lewis et al. 2024), but this specificity seems to develop over multiple years as most juveniles are more promiscuous than their adult counterparts (Abrego et al. 2009; Poland and Coffroth 2017; Liberman et al. 2024). From studies of sea anemones—a model for corals (Weis et al. 2008; Baumgarten et al. 2015)—it appears that the uptake of potential symbionts is indiscriminate, but only 'matching' Symbiodiniaceae avoid later expulsion or digestion via induced host immune suppression and potential arrest of phagosomal maturation (Voolstra et al. 2009; Mohamed et al. 2016; Jacobovitz et al. 2021). The exact molecules that signal friend rather than foe are unknown. Studies of glycan-lectin interactions have yielded conflicting results as to their importance in recognition (Parkinson et al. 2018; Tortorelli et al. 2022); other molecular patterns and recognition receptors warrant investigation (Baumgarten et al. 2015).

Experimentation with Symbiodiniaceae mutants has revealed that active photosynthesis is neither a prerequisite nor a requirement for symbiosis establishment, although it appears to be critical to the maintenance of healthy symbiont population densities (Mies et al. 2017; Jinkerson et al. 2022; Tran et al. 2024). Hosts and symbionts influence each other's cell division rates, though again, the mechanisms are not entirely clear, but data from thermal stress experiments indicate that control of nitrogen availability can regulate symbiont cell division rates (Tivey et al. 2020; Rädecker et al. 2023).

2.5 Evolutionary Considerations

Based on recent molecular clock analyses, the family Symbiodiniaceae emerged ~160 million years ago in the Jurassic Period (LaJeunesse et al. 2018). This timing coincides with the diversification of the precursors of modern-day scleractinian corals (Simpson et al. 2011). The implication is that corals and Symbiodiniaceae co-diversified and facilitated each others' success in the nutrient-poor tropics (Frankowiak et al. 2016). Because coral tissues form the key habitat for endosymbionts, selection drove host-specialization of generalist symbiodiniacean ancestors, leading to ecological diversification and speciation (Thornhill et al. 2014). Coral-algal mutualisms have persisted over expansive geological time and through multiple climatic changes (Wood 1998), though none so rapid as anthropogenic warming (Hoegh-Guldberg et al. 2007). It is therefore difficult to predict the adaptive capacity of these symbioses under current conditions.

Dinoflagellates possess unique genomes (reviewed by Lin 2011), and given the many aspects of Symbiodiniaceae ecology that distinguish them from other symbiotic microbial community members, it stands to reason that they harbor unique adaptive mechanisms (Wisecaver and Hackett 2011). Questions abound, such as whether Symbiodiniaceae genomes were reduced due to symbiosis, whether their most recent common ancestor was symbiotic or nonsymbiotic, and whether genome duplication events facilitated adaptive radiations. One hypothesis posits that Symbiodiniaceae are subject to resident genome syndrome, whereby prolonged intracellular associations reduce symbiont genome size and complexity over evolutionary time (González-Pech et al. 2019). However, many of the predicted features are lacking, such as strong genomic distinctions between free-living, facultative, and symbiotic species (Bhattacharya et al. 2024). Based on comparison to other free-living dinoflagellates, genome reduction appears to be common to all Symbiodiniaceae and likely took place in a free-living ancestor (Shah et al. 2024).

Recent theory posits that the retention of a facultative lifestyle among symbiotic Symbiodiniaceae provides a selective advantage over an exclusively symbiotic lifestyle (Bhattacharya et al. 2024). This 'stepping-stone' model considers the host as an incubator or bioreactor that facilitates symbiont specialization and diversification by giving optimal symbiont lineages a reproductive advantage. In culture and within host tissues, Symbiodiniaceae propagate clonally through asexual reproduction via mitosis (Tivey et al. 2020). Although sexual reproduction has not been directly observed, there is abundant molecular and cytological evidence that it takes place (Chi et al. 2014; LaJeunesse et al. 2014; Figueroa et al. 2021). Given the tight quarters within a host cell and the numerical dominance of a single symbiont genotype in most colonies, it seems likely that most reproduction occurs outside the host. Consequentially to this, symbionts are haploid for the majority of their life cycle (Santos and Coffroth 2003), and therefore mutations translate directly to phenotypes in the absence of a buffering allelic variant. When corals expel symbionts, the negatively buoyant cells sink and accumulate in the reef sediment (Littman et al. 2008), where they may encounter other sexually compatible cells expelled from other corals. Thus, a clonal lineage that establishes itself as the dominant symbiont in a colony gains a massive reservoir of identical cells that become available for sexual reproduction. Such selection within corals would favor genotypes that can establish symbioses easily, while selection without corals would favor genotypes that are most resilient in the free-living state (Bhattacharya et al. 2024). These successful genotypes are those most likely to be taken up by new hosts, creating a loop that prevents Symbiodiniaceae from becoming evolutionarily trapped in symbiosis while enabling both partners to codiversify.

Another complementary symbiont-focused theory seeks to explain why evolution has favored the maintenance of coral-algal partnerships that are susceptible to bleaching (Scott et al. 2024). The idea is that increasing seasonal symbiont loss during warm summer months—which occurs even in the absence of stress—coincides with host spawning (i.e., host reproduction) and the recruitment of symbiont-free coral larvae to the reef. Therefore, symbiont strains that are released from hosts at a higher rate represent a larger proportion of the free-living Symbiodiniaceae community, and thus have a greater chance of being acquired horizontally by the new coral larval recruits. This scenario would favor bleaching-susceptible partnerships when host life history involves high recruitment rates, slow adult growth rates, and low adult survivorship, whereas bleaching-resistant partnerships would be favored with low recruitment, fast growth, and high survivorship. Although the model is simple and intentionally excludes many important factors, it does predict general patterns that are consistent with data from Indo-Pacific reefs (Scott et al. 2024). These two recent models highlight that there is much to be gained from better incorporating the symbiont's perspective into coral holobiont evolutionary theory.

2.6 A Microbe's Microbiome

From decades of investigation, the accumulation of knowledge regarding the biology, ecology, physiology, and evolution of symbiodiniaceans is sufficient to recognize that these eukaryotes are quite distinct from other coral-associated microbes, especially bacteria (reviewed by Sweet et al. 2021; Voolstra et al. 2024). For example, symbiodiniaceans can be highly host-specific, whereas host bacterial community composition is determined by the environment to a greater extent (Roder et al. 2015; Ziegler et al. 2019; Dubé et al. 2021). Corals tend to be dominated by one clonal genotype of Symbiodiniaceae, whereas coral bacterial diversity exhibits order of magnitudes higher richness and evenness (Voolstra et al. 2024). Symbiodiniaceaens exchange nutrients with the host to the benefit of both partners, whereas many bacteria appear to be commensal. Of course, there are exceptions to all of these generalizations.

Symbiodiniaceae possess their own intracellular microbiome, with evidence for resident bacteria and viruses (Levin et al. 2017; Brüwer et al. 2017; Lawson et al. 2018; Howe-Kerr et al. 2023). It appears that some of these microbes are host-generalists that can be isolated from across a wide diversity of Symbiodiniaceae, while others may be more specific to particular algal species or environments (Table 2.2; Maire et al. 2021). There also appear to be both unique and common epibionts on the Symbiodiniaceae cell surface (Hill et al. 2024), which may be actively selected by algal metabolites that create a chemically enriched area around the photosymbiont (Bell and Mitchell 1972; Garrido et al. 2021), similar to the plant's rhizosphere effect (Katznelson 1946; Lv et al. 2023). Some bacterial taxa that are commonly detected within coral tissue samples may in fact associate with the symbionts rather than the host (Lawson et al. 2018; Maire et al. 2021; Hill et al. 2024).

The functions of most Symbiodiniaceae-associated microbes are yet to be elucidated. Cultured algae reared in antibiotic media often grow slower than their xenic counterparts (Soffer et al. 2008) and antibiotic treatment may favor the growth of different species in culture (Santos et al. 2001)—indications that bacteria likely contribute to Symbiodiniaceae metabolism. Hypothesized functional roles range from the purely trophic (bacteria as a nutrient source for heterotrophy; Jeong et al. 2012), to the complementary (bacteria as provisioners of metabolic pathways diminished or absent in Symbiodiniaceae; Ceh et al. 2013), to the tertiary (bacteria as mediators of environmental tolerance; Motone et al. 2020). Symbiodiniaceae-associated microbes have also been implicated in disease of both algae and corals, with preliminary findings suggesting that the progression of Scleractinian Coral Tissue Loss Disease (SCTLD) begins with a viral infection of susceptible symbiodiniaceans within the host tissue (Beavers et al. 2023).

2.7 Linking Past to Future

Scholars of Symbiodiniaceae—both new and experienced—can greatly benefit from reviewing earlier literature in the field. While methods have advanced and the appreciation of Symbiodiniaceae diversity has developed, many of the ideas surrounding how corals and their photosymbionts interact were established long ago. And yet, some current dogmas were enshrined early on and persist despite a lack of strong evidence; these assumptions may be ripe for reevaluation using modern approaches. Identifying such gaps requires careful attention to previous work. Given the extent to which the tools and taxonomy have changed, it is important to keep a few things in mind when plumbing the depths of older 'zooxanthellae' knowledge.

The most prominent historical shift in the literature has been the change in language reflecting the increasing recognition of diversity among Symbiodiniaceae over time. The first named species in the group, *Symbiodinium microadriaticum,* was taxonomically described in 1962 (Freudenthal 1962), a period when many thought that the genetic diversity of zooxanthellae was low (Taylor 1984). In the 1980s it was recognized that zooxanthellae derived from different host organisms had different properties (Schoenberg and Trench 1980a, b, c), and a handful of additional species were described (Trench and Blank 1987). In the 1990s and 2000s, rDNA sequencing revealed several deeply divergent lineages (referred to as "Clades" represented by letters; Rowan and Powers 1991, 1992) encompassing multiple putative species (ITS2 "types" represented by numbers; LaJeunesse 2001, 2005). At this point it was recognized that there were likely hundreds of Symbiodiniaceae species. In the 2010s, through additional species descriptions and revision, the genus '*Symbiodinium*' was split into multiple genera within the larger family Symbiodiniaceae, such that the modern genus *Symbiodinium* only constitutes members of the former 'Clade A' (LaJeunesse et al. 2018).

In much of the literature that has relied on the ITS2 marker, Symbiodiniaceae lineages are often referred to as "types" or "subtypes," generally with the "Clade" letter first and additional numbers and letters afterwards. These additional numbers and letters are arbitrary; they typically designate unique ITS2 variants originally identified through banding patterns in denaturing gradient gel electrophoresis (DGGE; LaJeunesse 2001), bacterially cloned PCR products (Apprill and Gates 2007), or next-generation amplicon sequencing (Arif et al. 2014; Hume et al. 2019). Importantly, the proximity of these numbers to each other within a genus ("Clade") is rarely indicative of evolutionary relatedness.

Table 2.2 The distribution of intracellular bacteria among Symbiodiniaceae

IntracellularBacterial Family	**Recovered From How ManySymbiodiniaceae Genera?**	*Breviolum*	*Cladocopium*	*Durusdinium*	*Fugacium*	*Gerakladium*	*Symbiodinium*
Alcanivoracaceae	1/6		✓				
Alteromonadaceae	3/6	✓	✓				✓
Balneolaceae	3/6		✓		✓		✓
Beggiatoaceae	3/6	✓	✓				✓
Beijerinckiaceae	6/6	✓	✓	✓	✓	✓	✓
Burkholderiaceae	4/6	✓	✓		✓	✓	
Caulobacteraceae	1/6			✓			
Corynebacteriaceae	3/6			✓	✓	✓	
Crocinitomicaceae	1/6		✓				
Cyclobacteriaceae	1/6		✓				
Hyphomicrobiaceae	6/6	✓	✓	✓	✓	✓	✓
Hyphomonadaceae	1/6		✓				
Moraxellaceae	1/6				✓		
Peudomonadaceae	1/6			✓			
Phycisphaeraceae	2/6		✓		✓		
Prevotellaceae	1/6				✓		
Rhizobiaceae	2/6				✓	✓	
Rhodobacteraceae	3/6		✓	✓			✓
Ruminococcaceae	1/6			✓			
Saprospiraceae	2/6		✓				✓
Sphingomonadaceae	3/6			✓	✓	✓	
Staphylococcaceae	2/6			✓		✓	

Some bacterial families appear unique to the interior of cells within specific Symbiodiniaceae genera, while others are more cosmopolitan. The table is based on one study of eleven algal cultures representing nine species and six genera, and only includes annotated bacterial taxa representing >3% of the intracellular prokaryotic community. Details will likely change as more experiments are performed. Data adapted from Maire et al. (2021)

For example, the *Breviolum* ITS2 lineage designated B1 is very distant on a phylogenetic tree from the B2 lineage, whereas B2 falls very close to B19 on the tree (see the phylogeny in Figure 6 of LaJeunesse 2005). Although these ITS2 types approximate species-level diversity, there is not necessarily a one-to-one correspondence between an ITS2 type and a species. Another example from *Breviolum*: while only one species is associated with the B2 ITS2 sequence, many species share the B1 ITS2 sequence (Parkinson et al. 2015). In the overwhelming majority of cases, one ITS2 sequence alone is not diagnostic (Davies et al. 2023). Because ribosomal DNA is multicopy (100s to 1000s of intragenomic copies) and the whole array is subject to concerted evolution (Dover 1994), distinct species lineages may share similar ITS2 sequence variants but in different proportions (e.g., both *Durusdinium glynni* (D1) and *D. trenchii* (D1a) share the same D1 variant, while *D. trenchii* alone possesses the additional 'a' variant (LaJeunesse et al. 2014; Wham et al. 2017). Much early confusion arose from studies that used cloning to isolate unique ITS2 sequence variants, which were often treated as representative of distinct species rather than considered as part of a larger fingerprint of variation within a single species' genome (Thornhill et al. 2007). Interpretation was even more fraught in samples from coral colonies with multiple co-ocurring symbiont species, especially if they happened to share similar ITS2 variants. This is where denaturing gradient gel electrophoresis and later amplicon sequencing provided an advantage in sorting intra- versus inter-genomic variation and resolving multiple fingerprints within a single colony.

The "alphabet soup" of ITS2 alphanumeric designations in older literature also produced inconsistencies and misidentifications. Sometimes, different research groups referred to the same variant by different names (e.g., C3 vs. C2 in LaJeunesse et al. 2003, van Oppen et al. 2001), or different variants by the same name (e.g., C1 (*Cladocopium goreaui*) vs. C1 (*Cladocopium proliferum*) in LaJeunesse 2001, Chakravarti et al. 2017). Sometimes, a different genetic marker was used, such as cp23S, but the sequence variant was assigned a code that was highly similar to an ITS2 type, such as B1 (ITS2) versus B184 (cp23S; Santos et al. 2004). Unfortunately, many misidentifications are still attached to entries in GenBank, causing no end of difficulty for individuals querying sequences against this database. A large proportion of GenBank entries haven't been updated to the new taxonomy, so they often misleadingly return '*Symbiodinium*' as the genus of the closest search result. Even the newer, curated SymPortal database generates novel designations that can be confused with older alphanumeric codes. There is no easy solution for these legacy issues; they will continue to be a problem even after most Symbiodiniaceae species have been assigned taxonomic binomials. Ongoing improvements to SymPortal aim to minimize such complications by depositing the defining ITS2 'type profiles' (sets of diagnostic ITS2 sequences) for species that have already been described by multi-locus genotyping approaches, combining the best of both worlds: taxonomic accuracy and fidelity with standardized high throughput symbiont typing.

Another naming issue relates to Symbiodiniaceae culture identities, which are typically uninformative at best and misleading at worst. For example, the culture 'Mf1.05b' was so named because it was isolated from a colony of *Montastrea faveolata* (now *Orbicella faveolata*). However, the cultured symbiont (*Breviolum minutum*) does not appear to be one of the *Breviolum* species that forms a stable symbiotic relationship with *O. faveolata* (Lewis et al. 2019). During the culturing process, the true symbionts, in absence of their host, were outcompeted and displaced by *B. minutum*. Contamination of algal cultures during and after isolation is common, and many of the species available in the global collection of Symbiodiniaceae cultures are not representative symbionts of the "hosts" initially ascribed to them (Santos et al. 2001). Such errors have propagated in culture metadata and they can be hard to track down, so it is advisable to maintain a healthy skepticism when such details are reported in the literature. Responsible researchers genotype their cultures before starting their experiments, typically reporting the ITS2 sequence and/or species name; if nothing else, this information should be reliable and consistent across studies using the same cultures.

2.8 Conclusions

This chapter has highlighted key biological aspects of dinoflagellates within the family Symbiodiniaceae–the primary photosymbionts of corals–and their essential role in the survival and health of coral reef ecosystems. Although the tremendous genetic diversity of Symbiodiniaceae is recognized, formal taxonomic species description remains challenging, as does reconciliation of taxonomy with high-throughput marker gene sequencing approaches. Accurate assessments of species diversity are a necessary prerequisite to any investigation of biodiversity, ecology, and evolution (i.e., adaptive capacity). Diversity within Symbiodiniaceae can enhance coral resilience to thermal stress and bleaching events, with evidence for a role of algal-associated microbes. However, the myriad ways ongoing environmental changes impact coral-algal associations have yet to be fully characterized, and the ability of such mutualisms to adapt to these changes remains difficult to predict. The availability of novel analytical tools, diminishing sequencing costs, and improvements towards methodological and analytical standardization should facilitate endeavors to better understand Symbiodiniaceae and the corals that depend upon them.

Dedication

This chapter is dedicated to the memories of James M. Lawrence (1946–2023), Dr. Scott R. Santos (1972–2024), and Dr. Robert K. Trench (1940–2021), all Symbiodineaceae popularizers and pioneers. They will be missed.

Appendix 1 All Validly Described Symbiodiniaceae Genera and Species as of 2024

Family-Level (n = 1)

Valid Names	**ITS2 Variants and Old/ Invalid Names**	**Most Relevant Taxonomic References**
Symbiodiniaceae	–	Fensome et al. (1993); LaJeunesse et al. (2018)

Genus-Level (n = 11–16)

Valid Names	**ITS2 Variants and Old/ Invalid Names**	**Most Relevant Taxonomic References**
Breviolum	“Clade B”	LaJeunesse et al. (2018)
Cladocopium	“Clade C”	LaJeunesse et al. (2018)
Durusdinium	“Clade D”	LaJeunesse et al. (2018)
Miliolidium	“Foraminifera Clade D”	Pochon and LaJeunesse (2021)
Effrenium	“Clade E”	LaJeunesse et al. (2018)
Freudenthalidium	“Clade F” (Fr3)	Nitschke et al. (2020)
Fugacium	“Clade F” (Fr5)	LaJeunesse et al. (2018)
Gerakladium	“Clade G”	LaJeunesse et al. (2018)
Halluxium	“Clade H”	Nitschke et al. (2020)
Philozoon	“Temperate Clade A,” “Temperate-A,” “Mediterranean A,” “A-prime,” “A^{I}”	Geddes (1882); LaJeunesse et al. (2022)
Symbiodinium	“Clade A”	Freudenthal (1962); LaJeunesse et al. (2018)
“*Symbiodinium*” Clade Fr2	“Clade F” (Fr2)	Pawlowski et al. (2001)
“*Symbiodinium*” Clade Fr4	“Clade F” (Fr4)	Pawlowski et al. (2001)
“*Symbiodinium*” Clade G	“Foraminifera Clade G”	Pochon et al. (2001)
“*Symbiodinium*” Clade I	“Clade I”	Pochon and Gates (2010)
“*Symbiodinium*” Clade J	“Clade J”	Yorifuji et al. (2021)

Species-Level (n = 44)

Valid Names	**ITS2 Variants and Old/ Invalid Names**	**Most Relevant Taxonomic References**
Breviolum aenigmaticum	–	Parkinson et al. (2015)
Breviolum antillogorgium	B1	Parkinson et al. (2015)
Breviolum dendrogyrum	B1, B1k	Lewis et al. (2019)
Breviolum endomadracis	B7	Parkinson et al. (2015)
Breviolum faviinorum	B1, B14, B14a	Lewis et al. (2019)
Breviolum meandrinium	B1, B20	Lewis et al. (2019)
Breviolum minutum	B1, B1-1; “*S. pulchrorum*”	LaJeunesse et al. (2012a)
Breviolum pseudominutum	B1; “*S. bermudense*”	Parkinson et al. (2015)
Breviolum psygmophilum	B2	LaJeunesse et al. (2012a)
Cladocopium goreaui	C1	Trench and Blank (1987)
Cladocopium infistulum	C2	Lee et al. (2020)
Cladocopium latusorum	C1c, C1b-c, C42, C42a, C42b, C1c-ff, C1c-42-ff	Turnham et al. (2021)
Cladocopium madreporum	C40	Butler et al. (2023)
Cladocopium pacificum	C1d, C1d-t	Butler et al. (2023)
Cladocopium patulum	C3u	Butler et al. (2023)
Cladocopium proliferum	C1, C1-Acro	Butler et al. (2023)
Cladocopium sodalum	C3 (LaJeunesse et al. 2003), C2 (van Oppen et al. 2001)	Butler et al. (2023)
Cladocopium thermophilum	C3, C3-Gulf	Hume et al. (2015)
Cladocopium vulgare	C1, C1-Psam	Butler et al. (2023)
Durusdinium boreum	D15	LaJeunesse et al. (2014)
Durusdinium eurythalpos	D8, D8-12, D12-13, D13	LaJeunesse et al. (2014)
Durusdinium glynni	D1	Wham et al. (2017)
Durusdinium trenchii	D1a, D1-4	LaJeunesse et al. (2014)
Effrenium voratum	E1; “*S. californium*”	Jeong et al. (2014)
Freudenthalidium endolithicum	F3.8, Fr3.8	Nitschke et al. (2020)
Freudenthalidium heronense	F3.7, Fr3.7	Nitschke et al. (2020)

Valid Names	ITS2 Variants and Old/ Invalid Names	Most Relevant Taxonomic References
Fugacium kawagutii	F1, Fr5	Trench and Blank (1987)
Gerakladium endoclionum	–	Ramsby et al. (2017)
Gerakladium spongiolum	–	Ramsby et al. (2017)
Halluxium pauxillum	H7	Nitschke et al. (2020)
Miliolidium leei	D1.1	Pochon and LaJeunesse (2021)
Philozoon actinarium	A19	LaJeunesse et al. (2022)
Philozoon adriaticum	–	LaJeunesse et al. (2022)
Philozoon anthopleurum	–	LaJeunesse et al. (2022)
Philozoon balanophyllum	–	LaJeunesse et al. (2022)
Philozoon colossum	–	LaJeunesse et al. (2022)
Philozoon geddesianum	–	LaJeunesse et al. (2022)
Philozoon medusarum	–	LaJeunesse et al. (2022)
Philozoon paranemonium	–	LaJeunesse et al. (2022)
Symbiodinium microadriaticum	A1; "*S. microadriaticum* subsp. *microadriaticum*"	Freudenthal (1962)
Symbiodinium natans	–	Hansen and Daugbjerg (2009)
Symbiodinium necroappetens	A13, A1.1; "*S. cariborum*," "*S. microadriaticum* subsp. *condylactis*"	LaJeunesse et al. (2015)
Symbiodinium pilosum	A2; "*S. corculorum*," "*S. meandrinae*"	Trench and Blank (1987)
Symbiodinium tridacnidorum	A3-Pacific, A3*, A3a, A3x, A6	Lee et al. (2015)

Additional details include the majority ITS2 sequence(s) known to be present in each taxon's genome, old/invalid names, and the 1–2 most relevant taxonomic references. Updated from Table 2.1 in Davies et al. (2023). Note that "*Symbiodinium linucheae"* is no longer considered valid because the type specimen in the original description was a living culture (now lost) rather than a permanent deposition, which is not acceptable under the International Code of Nomenclature (ICN) for Algae, Fungi, and Plants (Article 8.4).

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3 Bacteria and Archaea Within Coral Reef Ecosystems

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Abstract

Coral reefs are biodiverse ecosystems which rely on microorganisms for nutrient cycling, immune benefits, and contributing to general ecosystem function and stability. Microorganisms reside in diverse coral reef habitats including the water column, sediments, reef surfaces, and in association with reef life. This chapter focuses on the prokaryotic (Bacteria, Archaea) members of the reef community. First, a history of major discoveries and advancements about Bacteria and Archaea in reef environments is presented, alongside the significance of these findings. Then, attention to bacterial and archaeal communities residing in reef water, sediment, and select reef organisms (corals and fish) is presented. Within each habitat, there is a focus on (a) general trends in the diversity, composition, and functions of microorganisms, (b) dynamics of the microorganisms and connection to reef health and (3) outlook and future research needs. Overall, there has been considerable progress observing and inventorying Bacteria and Archaea in reef environments. Many opportunities exist to further this understanding into specific mechanisms surrounding the role of specific microorganisms and microbial communities in host and reef ecosystem biogeochemistry, health, resilience and applications of this knowledge into conservation and restoration activities.

Keywords

Prokaryote · Bacteria · Archaea · Coral reef · Fish
Habitat · Nutrient · Immunity

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3.1 Introduction

Microorganisms belonging to the kingdoms Bacteria and Archaea are prominent members of biodiverse and economically important coral reef ecosystems. They are the most abundant cellular life in this ecosystem and reside in association with all forms of reef life as well as in the water column, on habitat surfaces and within the sediment. In these diverse reef compartments, microbes comprise hundreds of thousands to billions of cells per ml, reef organism or sediment area (Fig. 3.1). These cells contribute to energy production, nutrient recycling and degradation and protection of reef life against pathogens (Bourne et al. 2016). Coral reef prokaryotes contribute substantial biodiversity to reefs. A recent study estimated that 2.8 million discreet prokaryote taxa (amplicon sequence variants, ASVs) associate with reefs (Galand et al. 2023), which exceeds current world's estimates of prokaryotic diversity (0.8–1.6 million taxa) (Louca et al. 2019). Coral reef microorganisms are abundant and diverse on reefs and are clearly pillars of coral reef ecosystems, spearheading major reef processes and contributing to ecosystem stability and resilience.

Because there are a cornucopia of review papers focusing on coral reef microbiology (largely focused on the stony corals), I attempted to bring a different perspective to this topic. First, I offer a historical perspective into major discoveries and advancement in the field. Those occurring in the years 2002 and beyond I was fortunate enough to read 'hot off the press' during my career. Secondly, this chapter attempts to be holistic towards the different coral reef habitats supporting Bacteria and Archaea. I focused on active areas of research in my laboratory: reef water, sediments, corals and reef fish. Sponge microbiomes are also important on reefs and play a large role in maintaining reef water quality but are not included here. I direct you to a number of excellent reviews and studies of reef sponge microbiology (Cleary et al. 2019; Fiore et al. 2020; Hentschel et al. 2012; Pita et al. 2018; Thomas et al. 2016b). This chapter also discusses the

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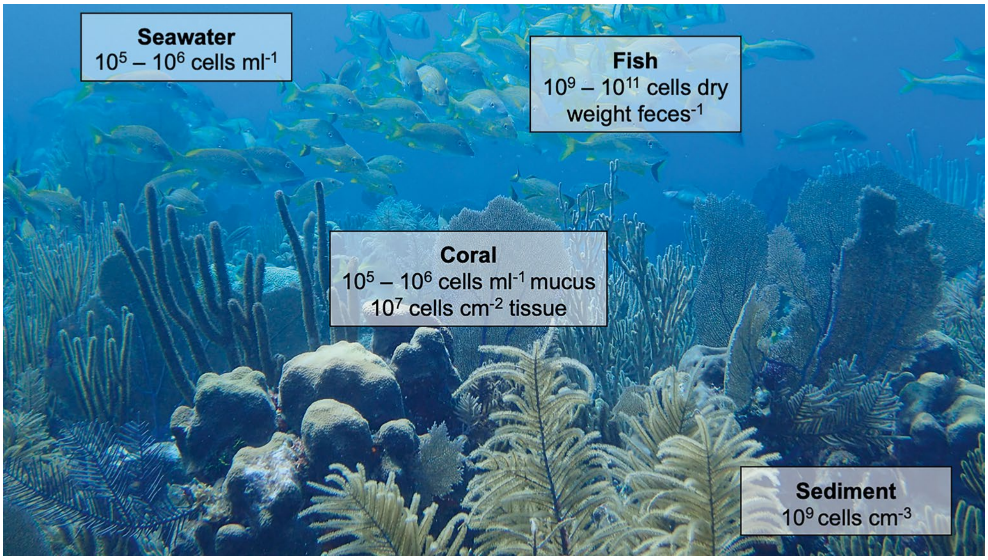

Fig. 3.1 Microorganisms are abundantly associated with coral reefs. Abundances of organisms are approximate and are based on reports from previous studies: seawater (Apprill et al. 2021; Weber and Apprill 2020), fish (Smriga et al. 2010), coral mucus (Ducklow and Mitchell 1979a; Koren and Rosenberg 2006), coral tissue (Wegley et al. 2004) and sediment (Schöttner et al. 2011). Photograph from Jardines de la Reina, Cuba

role of microorganisms in reef ecosystem health, which is a critical subject for declining reefs. Finally, each section identifies key needs for advancing knowledge and understanding of coral reef prokaryotes.

3.2 Major Observations, Discoveries, and Advancements in Coral Reef Microbiology

Advancements and major discoveries in coral reef microbiology largely follow three general eras. The first includes early observations and discoveries (1955–2006). This was followed by an era of technology-enabled discoveries, which are often attributed to the application of newly available cultivation-independent based approaches (2001—present). These two eras coincided with a period (2002—present) examining the roles of microbes in coral and reef health, which integrated culture and cultivation-independent approaches and took advantage of integrative ‘omics and cell-cell visualization techniques. There are many noteworthy and exciting events over the last ~65 years, and a few of the prominent events are highlighted in Fig. 3.2.

3.2.1 Early Observations (1955-2006)

The earliest discoveries in coral reef microbiology resulted from microscopic and cultivation-based observations. The first evidence of bacteria colonizing surfaces in a coral reef environment was made by Odum and Odum (1955). Bacteria were first isolated from corals by DeSalvo (1969) during a study in Kaneohe Bay, Hawaii, and these bacteria provided the first evidence that bacteria can break down coral skeletons. Work by Sara (1971) showed that reef sponges harbor cyanobacterial symbionts. In 1973, Sorokin demonstrated that reef water and sand bacteria were abundant on reefs, with higher estimates in sands, and described the first major role of bacteria in the reef food web. Specifically, Sorokin showed that filter feeding organisms including sponge and coral remove bacteria from reef water in a density-dependent manner (Sorokin 1973a, b).

Microbial associations in sponges were further and more prominently described in 1978 (Wilkinson 1978). Bacteria were first discovered within coral mucus by Ducklow and Mitchell in 1979. Early histological work by Ester Peters documented the first bacteria within the tissues of apparently healthy coral (Peters 1984). In 1985, reef fish gut microorganisms were first observed and shown to host giant bacteria (Fishelson et al. 1985). In 2006, Ritchie used cultivation-based approaches to show that coral-associated bacteria could provide antibacterial activities, which have the potential to benefit corals (Ritchie 2006). There were many observations and cultivation of microorganisms associated with diseased corals during this time, and I refer you elsewhere for this timeline (Bruckner 2015).

3.2.2 Technology-Enhanced Discoveries (2001-Present)

Rohwer and colleagues applied the first cultivation-independent approach to corals, and demonstrated their high

Early Observations

1955: Bacteria colonize reef surfaces (Odum & Odum)
1969: Bacteria first isolated from corals (DeSalvo)
1971: Sponges have prokaryotic symbionts (Sara)
1973: Role of bacteria in reef food web (Sorokin)
1979: Bacteria reside within coral mucus (Ducklow & Mitchell)
1984: Bacteria reside within healthy coral tissue (Peters)

Technology-enhanced Discoveries

2001 & 2002: Coral harbors high bacterial diversity & host specificity (Rohwer et al.)
2004: Coral-nitrogen fixing cyanobacteria symbiosis identified (Lesser et al.)
2004: Coral-associated archaea discovered (Kellogg; Wegley et al.)
2006: Taxon-specific imaging of bacteria within corals (Ainsworth et al)
2007: Application of metagenomics to coral-microbes (Kelly et al.)
2009: Horizontal transmission of bacteria into corals (Apprill et al.)
2010: Recognition of microbiomes in reef fish microbiome study (Smriga et al.)
2010: Revised estimate of coral-associated microbial diversity using amplicon sequencing (Sunagawa et al.)
2012: Vertical transmission of bacteria into corals (Sharp et al.)
2012: Microbial manipulation of corals (planulae) (Apprill et al.)
2013: Application of NanoSIMS to coral-prokaryote symbiosis (Ceh et al.)
2017: Metagenome-assembled genome and single cell sequencing of coral-bacteria (Neave, Mitchell et al.)
2019: Evidence for the coral ecosphere (Weber et al.)

Reef Health

2002: Bacterial Bleaching Hypothesis (Rosenberg & Ben-Haim)
2007: Evidence against the Bacterial Bleaching Hypothesis (Ainsworth et al)
2006: Coral Probiotic Hypothesis (Reshef et al)
2016: Reef Microbialization (Haas et al.)
2017: Beneficial Microorganisms for Corals (BMC) (Peixoto et al.)
2017: Coral microbiome flexibility (Ziegler et al.)
2017: Reef water microbes as diagnostic indicators for reefs (Glasl et al.)
2019: Coral-bacteria provide bleaching resistance (Rosado et al.)
2021: Coral microbiome manipulation alleviates bleaching and mortality (Santoro et al.)
2023: Enhancing coral-bacteria cultivation for probiotics development (Modolon et al.)
2023: Microbial genomic & metabolomic coupled reef health indicators (Becker et al.)
2024: Field application of coral probiotics (Delgadillo-Ordoñez et al.)

Fig. 3.2 Timeline of major coral microbiology observations, discoveries and advancements. Events are a selection from many notable and important events outlined in this chapter

bacterial diversity (Rohwer et al. 2001) and host-bacterial specificity across geographic distance (Rohwer et al. 2002). I fondly remember reading these two articles in the historic library of the Marine Biological Laboratory in Woods Hole, MA during my time as a student in the ‘Microbial Diversity’ course. These two articles caused me to pivot my graduate studies from open ocean to coral-associated microorganisms, with the anticipation that this field of coral reef microbiology would further grow with the exciting advances in DNA sequencing and microscopy. Shortly following the work by Rohwer and colleagues, Lesser discovered nitrogen fixing cyanobacteria symbiotic within coral tissues (Lesser et al. 2004). In 2004 and within the same issue of the journal *Marine Ecology Progress Series* was the coordinated finding that corals host Archaea (Kellogg 2004; Wegley et al. 2004), which further demonstrated that marine archaea do not solely reside in hydrothermal vents and other extreme environments. In 2006, Ainsworth and colleagues contributed the first taxon-specific imaging of coral-associated bacteria using fluorescent in situ hybridization probes (Ainsworth et al. 2006). In 2007, Wegley and colleagues applied the first next-generation sequencing methods to corals, to examine the microbiome of *Porites astreoides* using metagenomic-based techniques (Wegley et al. 2007). This was followed by the first amplicon sequencing methods applied to coral reefs in 2010 by Sunagawa and colleagues, which described high diversity of coral-associated bacteria (Sunagawa et al. 2010). Since that time, hundreds of studies have utilized next-generation sequencing to explore the diversity, composition and symbiotic nature of coral-associated microbes.

My own work was the first to demonstrate horizontal transmission of microbes to coral (Apprill et al. 2009). My colleagues and I applied this knowledge to a microbial manipulation experiment to demonstrate specificity of bacterial uptake by corals (Apprill et al. 2012). Vertical transmission of bacteria into corals was shown in 2012 by Sharp and colleagues (Sharp et al. 2012). In 2013, the first application of nanoscale resolution secondary ion mass spectrometry investigations (NanoSIMS) on corals was conducted, which showed nitrogen transfer from bacteria into host and algal symbionts (Ceh et al. 2013). Metagenome-assembled genomics (MAG) and single-cell sorting and sequencing were first applied to examine coral-*Endozoicomonas* relationships in 2017 (Neave et al. 2017a). Advancements in sequencing of low microbial biomass paved the way for the discovery of the 'coral ecosphere', or the recognition that corals harbor unique, specific-specific microbial communities in the surrounding waters with genomic features suited for host interaction (Weber et al. 2019).Cultivation-independent approaches continue to be applied to coral reef environments, and much of the information in this chapter is taken from studies during this ongoing era.

3.2.3 The Role of Microbes in Coral and Reef Health (2002-Present)

During the time of rapid coral-microbial discovery spurred by cultivation-independent approaches, the Bacterial Bleaching Hypothesis was developed. This posited that bacterial pathogens can cause bleaching in corals, specifically in the *Oculina patagonica*-*Vibrio shiloi* model (Rosenberg and Ben-Haim 2002; Rosenberg and Falkovitz 2004) (at the time bleaching by other mechanisms including thermal stress was well-established). However, work by Ainsworth and colleagues refuted this hypothesis (Ainsworth et al. 2007). Interestingly, the coral probiotic hypothesis emerged during this time which suggested that the *O. patagonia* developed resistance to *V. shiloi* infection due to coral microbiome-based pathogen resistance (Reshef et al. 2006). This work paved the way for further significant interest and research in actively manipulating these coral-associated beneficial microbes (Beneficial Microorganisms for Corals, BMC) by Peixoto and colleagues (Peixoto et al. 2017). Research led by Rosado combined cultivation and cultivation-independent approaches to show the first experimental evidence of coral-bacteria causing bleaching resistance (Rosado et al. 2019). In the timeframe of 15 years, coral-bacteria went from being a primary cause of bleaching to a solution to prevent bleaching. Advancements with probiotics continue, including showing evidence for a role in heat stress tolerance (Santoro et al. 2021), technology-enhanced coral-probiotic cultivation (Modolon et al. 2023) and successful field-based application of coral probiotics (Delgadillo-Ordoñez et al. 2024) which are all important efforts designed to enhance coral health.

The recognition of microbes in reef health has extended beyond corals to other reef environments and organisms. Butterflyfish were introduced as possible coral disease vectors in 2009, due to their feeding on stony corals and potential for microbial transfer between colonies (Raymundo et al. 2009), and algae as reservoirs of coral disease-associated bacteria in 2011 (Barott et al. 2011). Acknowledgement of the link between benthic features, dissolved organic carbon and enhanced pathogens initially by Nelson et al. (2013) led to the development of the microbialization hypothesis for reefs, which was documented by Haas and colleagues on a global scale in 2016 (Haas et al. 2016). During this similar time, reef water microbes were also growing in recognition as diagnostic indicators of reef water quality and reef health in 2017 (Glasl et al. 2017). More recently (2023), reef water microbial genomic and metabolomic health indicators were introduced, further paving the way for using non-invasive sampling of reef water to monitor reef ecosystem health (Becker et al. 2023).

3.3 Reef Water Microbiome

3.3.1 General Features

The water overlying the reef is a dynamic habitat which supports a diverse and abundant community of microorganisms. Cells are often free-ranging (pelagic or planktonic). Cells are also particle-associated, adhering to neutrally buoyant detritus including mucus from corals and organisms. Reef water prokaryotes typically comprise around 500,000 to over a million or more cells per milliliter of water (Apprill et al. 2021; Weber and Apprill 2020) (Fig. 3.1). This community of reef water microbes is diverse in composition, and diversity is typically accounted for using sequencing-based estimations. Depending on the resolution of sequence data, microbial richness (number of microbial taxa) is on the order of 100–450 taxa (Apprill et al. 2021; Weber et al. 2020; Ziegler et al. 2016). Studies differ in observations of how reef water microbial community richness compares to benthic organisms (Galand et al. 2023; Sunagawa et al. 2010). Most studies agree that reef water microbial communities show exceptionally low beta diversity compared to corals and other reef organisms (Apprill et al. 2016; Galand et al. 2023; Glasl et al. 2019), suggesting that there are governing ocean and reef principles dictating reef water microbial cell growth and community composition. Surface water microbiomes tend to be less diverse compared to benthic or reef-depth waters from shallow coral reefs, with this difference attributed to unique taxa residing in the benthos that utilize benthic-produced organic carbon to support growth. Growth rates of reef water microbial cells are generally 0.5–1 or more divisions per day, but this is understudied (McNally et al. 2017; Weber and Apprill 2020).

Depending on the geographic location, about one-half to three-fourths of the reef water column microbial community is composed of photosynthetic cyanobacteria (Frade et al.

2020; Weber and Apprill 2020). These cells include *Prochlorococcus* and *Synechococcus*, which generally divide nightly (Becker et al. 2020; Weber and Apprill 2020), and sustain a steady input of new carbon into reef environments. The identity of these cells generally reflects the prevailing oceanographic and environmental conditions, with *Prochlorococcus* prevalence reflecting oligotrophic conditions such as oceanic (non-reef water) input and *Synechococcus* reflecting more coastal-based nutrient conditions. These differences are attributed to their differential nitrogen requirements and distribution of nitrogen forms and availability (Moore et al. 2002). One example of these distribution differences is Kaneohe Bay, Hawaii; *Synechococcus* is abundant in the bay with *Prochlorococcus* absent and the trend reverses near the edge of the embayment (Cox et al. 2006; Yeo et al. 2013). *Synechococcus*, which are about twice the size in diameter compared to *Prochlorococcus* (Wei et al. 2019), can be selectively consumed by corals and likely other reef organisms, providing a reliable food source in this environment (McNally et al. 2017). *Prochlorococcus* growth can also be influenced by specific reef organisms (Weber et al. 2022). Additionally, *Prochlorococcus* abundances can relate to temperature, salinity, ammonium and dissolved organic carbon concentrations in reef environments (Becker et al. 2024; Frade et al. 2020; Glasl et al. 2017). The nitrogen fixing cyanobacterium *Trichodesmium* are often visible to divers in the water column, typically as puff and tuff-based aggregations. However, they are generally low in relative abundance compared to *Prochlorococcus* and *Synechococcus* (Weber and Apprill 2020).

Reef water cells lacking chlorophyll content often comprise about half of the microbial community. This community is commonly dominated by taxa referred to as oligotrophs which are also featured in offshore tropical waters, including SAR11 (*Pelagibacter*), SAR86, "*Candidatus* Actinomarina" and members of the AEGEAN-169 marine group (Apprill et al. 2021; Weber et al. 2020). Copiotrophic (higher nutrient necessitating) microbial taxa also reside in reef waters, including members of the Rhodobacteraceae, Flavobacteraceae, Verrucomicronbiales, Alteromondales, Vibrionales and archaeal Thermoplasmatales (Apprill et al. 2021; Weber and Apprill 2020). These taxa are often identified in coastal, temperate and other non-reef habitats, suggestive of their affinity for more multifaceted environments whose growth may depend on substrates associated with land, organisms or sediment.

3.3.2 Factors Influencing Reef Water Microbial Dynamics

Biogeography is a major influence on the composition of reef water microbial communities. Indeed, reef water microbial communities are generally most similar within a reef site and variation between communities grows with distance and contrasting ocean features (Becker et al. 2023; Ma et al. 2022). Studies have demonstrated that microbial communities are structured by biogeographic patterns representing close distant but contrasting reef environments, such as between lagoons and barrier reefs (McCliment et al. 2012). Additionally, microbial communities are also distinguished by larger distances including between islands in a larger chain (Apprill et al. 2021; Salerno et al. 2016) or even distance alone when compared across ocean basins (Ma et al. 2022).

The macro-organismal reef community impacts the structure and dynamics of reef water microorganisms (Kelly et al. 2014). Growth of some heterotrophic cells are enhanced by exudates from benthic organisms, causing microbialization and DOC drawdown on some reefs (Haas et al. 2016). Exposure to macroalgae, crustose coralline algae and coral exudates generally increases the growth rate of picoplankton (Haas et al. 2013; Nelson et al. 2013). Coral exudates enhance the relative abundance of the Rhodobacteraceae taxa *Mariviens*, HIMB11 and unclassified taxa as well as Cellvibrionales and *Alteromonas* (Weber et al. 2022). There is also evidence that macroalgae may promote the growth of pathogens and microbes with enhanced virulence factors, including Vibrionaceae and Pseudoalteromondaceae (Nelson et al. 2013). Experiments have noted depletions of some oligotrophic taxa including SAR86, SAR324 marine group B, SAR116 and Marine Group 2 archaea (Poseidoniales) in the presence of coral exudates (Weber et al. 2022). In field surveys, coral abundance and richness has been correlated with reef water microbiomes (Apprill et al. 2021; Becker et al. 2023; Weber et al. 2020). There is less known about the influence of fish on reef water microbial communities, and they presumably impact communities through excretion and feeding. Ephemeral events, such as coral spawning can also influence reef water microbial community structure (Apprill and Rappé 2011; Glud et al. 2008; Patten et al. 2008).

As expected, environmental and water quality features influence reef water microbial communities. A study of the Great Barrier Reef (GBR) microbial communities over 16 months identified temperature, season, total suspended solids, organic carbon and chlorophyll *a* as related to microbial communities (Glasl et al. 2019). A larger spatial scale study of GBR surface microbial communities identified temperature and nutrient dynamics to explain over 40% of the microbial community variations and identified a Flavobacteriaceae taxa as diagnostic for reef degradation (Frade et al. 2020). Further, biogeographic, benthic and nutrient properties defined microbial communities across the 500 km Florida barrier reef (Becker et al. 2023). Following hurricane and disease-disturbed reefs over time, Becker and colleagues identified increased ammonium coinciding with a decline in *Prochlorococcus* and increase in heterotrophic bacteria, suggesting a type of microbialization fueled by disturbance (Becker et al. 2024).

3.3.3 Outlook and Future Research Needs for Coral Reef Water Microbiology

There is growing evidence that reef water microorganisms serve as diagnostic indicators of reef condition. The microbial communities are cumulative in their ability to integrate biogeographic, environmental/water quality and reef features, and they could play a larger role in documenting reef health conditions. Development of reef water microbes as health and environmental proxies for reefs is rapidly emerging with now over a dozen studies examining reef water microbial dynamics (Becker et al. 2020, 2023; Frade et al. 2020; Glasl et al. 2017, 2018, 2019; Kelly et al. 2014; Weber and Apprill 2020). Further development of reef water microbes as health sensors necessitates additional time-series studies in diverse geographic regions. When conducted alongside environmental and reef surveys, this could allow us to better constrain microorganisms reflective of specific conditions or features, allowing us to identify specific microorganisms diagnostic of reef condition (Apprill et al. 2023). Additionally, this type of knowledge will pave the way for new hypotheses about the interaction between reef water microbes and specific reef or ocean processes. Technological needs to assist this goal include development of cost-effective autonomous sampling devices as well as databases of typical reef microbes and community features to ease interpretation of trends and to relate microbial indicators to reef health.

There are also many opportunities to expand current knowledge about reef water microorganisms through targeted studies. Nothing is known about mesophotic reef water microbial communities, which likely play a larger role in reef processes in these low light environments. There is also an opportunity to identify if and how fish and other pelagic and sometimes transitory organisms impact reef water microorganisms and dynamics. Lastly, there have been few metagenomic studies of reef water (Becker et al. 2023; Kelly et al. 2014). Additional investigations could help reveal the metabolic potential of communities. For example, do reef oligotrophic taxa have unique capabilities of residing in this biodiverse habitat? What is the potential for pelagic, copiotrophic taxa to exhibit pathogenicity and contribute to coral and other diseases?

3.4 Reef Sediment Microbiome

3.4.1 General Features

Sediment varies in abundance and composition around coral reefs (Bothner et al. 2006; Whinney et al. 2017). The sediments of most coral reefs are sandy and permeable and provide a rich habitat to microorganisms (Alongi et al. 2007). The composition of reef sediments varies and is influenced by the local conditions. Some reef sediments are carbonate, formed from calcifying organisms including corals. Reef sands can also be silicate-rich and harbor terrestrial deposits, which will impact the grain size, porosity and permeability (Rasheed et al. 2003). The geophysical features of the sediment impacts organic matter retention and the types of microbial communities that reside within.

The microbiomes of reef sediments are understudied, which may be related to the fact that sediment distributions can vary widely within and between reef habitats. In the Gulf of Aquaba, Red Sea, cell densities range from 1.5–3 × 10^9 cells cm^{-3} (Schöttner et al. 2011) (Fig. 3.1). Sediments microbiomes are comprised of both bacteria and archaea and are generally highly diverse (Dong et al. 2023), hosting more taxa compared to seawater, corals, sponges and macroalgae (Glasl et al. 2019). Diversity patterns are generally similar within different sediment depths, but the taxonomy of the community does differ alongside biogeochemical gradients (Schöttner et al. 2011). Studies report Rhodobacteraceae, Acidobacteriales and the ammonia oxidizing archaea *Nitrosopumilus maritimus* - among other taxa - residing within reef sands (Gaidos et al. 2011; Schöttner et al. 2011). Microbial community similarity amongst biogeographically distant sands does exist, such as between Florida and Fiji reefs, suggestive of larger governing properties (León-Zayas et al. 2020). Community composition can also vary with seasonal-related dynamics, for regions like the Gulf of Aqaba with seasonality patterns of winter shallow mixing and upwelling of higher nutrient waters (Schöttner et al. 2011), but they are more stable in areas like the Great Barrier reef which experience fewer seasonal dynamics (Glasl et al. 2019).

Microorganisms in reef sediments play a major role in nutrient transformations. In Great Barrier Reef carbonate sediments, bacterial nitrogen fixation in surface sediments supplies high ammonium turnover, attributed to an active community of nitrifiers (Capone et al. 1992; Rusch and Gaidos 2013). Metagenomic studies have suggested that most sediment microbes are capable of aerobic respiration, and there is evidence that they can rely on other terminal electron acceptors under anoxic conditions including nitrate, nitrite, sulfate and gases such as nitric acid and nitrous oxide (Dong et al. 2023).

3.4.2 Connection of Reef Sediments to Reef Health

Although understudied, microorganisms in sediments are generally thought to play a major role in reef health. Due to the biogeochemical complexity of sediments, they have the potential to serve as reservoirs for pathogens. Stony coral tissue loss disease, now widespread in the Caribbean, is transmissible through reef sediments (Studivan et al. 2022) and these sediments can harbor relatives of pathogens (Rosales et al. 2023). Dredging activities related to port construction and maintenance are therefore of concern for introducing novel microbes and possibly pathogens to reefs (Miller et al. 2016). Further, reef sediments may play a useful role in anthropogenic activity diagnostics. A study of Taiwanese sediments detected enhanced human-related microbes related to rainfall events, suggesting runoff-based contamination of the reef sediments (Wang and Liu 2023).

Metagenomic studies in the South China Sea have suggested that as many as 10% of microbial cells may harbor antibiotic resistance genes, including aminoglycoside, tetracycline and fosfomycin (Dong et al. 2023). This high resistance is attributed to the generally high microbial species diversity harbored in reef sediments, and thus reef sediments could be thought of as a general reservoir of novel biological traits, which may or may not be beneficial to reefs. Overall awareness and sensitivity towards this biodiversity reservoir is essential to the management and conservation of reef ecosystems.

3.4.3 Outlook and Future Research Needs for Sediment Microbiology

Despite enhanced collections and microbial data on reefs, reef sediment microbiomes remain underexplored. For example, the recent Tara Pacific expedition produced extensive data on plankton and reef organism microbiomes, but not sediment (Galand et al. 2023). Globally comparable data should extend beyond diversity and taxonomy descriptions, and include metagenomic analysis of these metabolically diverse communities. Further, given the underexplored nature of reef sediments, focus on cultivation is necessary to fully examine the metabolic activities and uncover specific roles of these cells within the biogeochemically diverse reef sediments. Lastly, diversity and taxonomic descriptions (e.g., amplicon sequencing) are still needed to examine the extent that sediment microbes can reflect reef ecosystem health and other episodic events, and time series type analyses may be particularly ideal for developing this framework.

3.5 Coral-Associated Microbiome

3.5.1 General Features

Corals are anthozoans, the framework building animals on coral reefs, providing habitat for diverse reef life. There are over 6000 species of anthozoans generally within the major stony corals and octocoral groupings. Stony corals are composed of aragonite skeletons which record historical seawater temperature and chemical conditions in their skeletons. Octocorals, which includes soft corals, sea pens and gorgonians, have a soft matrix-like skeleton, often of calcareous material. Stony coral microbiomes have been examined in numerous studies and review articles (e.g., Ainsworth et al. 2015; Bourne and Webster 2013; Bourne et al. 2016; Hernandez-Agreda et al. 2017) but octocoral microbiomes have received historically less but recently growing attention (Keller-Costa et al. 2022; Reigel and Hellberg 2023; van de Water et al. 2018).

Coral microbiomes are distinguished by anatomical region. This includes the near coral seawater microbiome as well as the major anatomical regions including surface mucus, tissue and skeleton. The near-coral microbiome has been investigated in several studies using small volume samplings in close proximity to corals (Ochsenkühn et al. 2018; Tout et al. 2014; Walsh et al. 2017; Weber et al. 2019). This has resulted in reports of enhanced copiotrophic taxa residing within centimeters of the coral surface, including *Endozoicomonas, Bermanella, Alteromonas, Psychorbacter, Erythrobacter, Marinobacter*, compared to the overlying water comprised of the more oligotrophic *Prochlorococccus*, SAR11 and SAR86, NS5 and NS6 marine groups and "*Candidatus* Actinomarina" (Weber et al. 2019). This near-coral environment is termed the 'ecosphere' or 'aura-biome' because it is ecologically distinct compared to the overlying seawater, likely due to the release of dissolved organic matter from the coral surface (Walsh et al. 2017; Weber et al. 2019). Ecosphere-residing cells are generally copiotrophic taxa enriched in genes for interactions, biofilm formation, and metabolite uptake and exchange (Tout et al. 2014; Weber et al. 2019). To my knowledge, these ecospheres are readily associated with stony corals but have not yet been investigated for octocorals.

Corals secrete mucus which covers their surface and functions as a trap for sediment and particles. The mucus is composed of protein, polysaccharides and lipids and the composition and rate of production varies by species (Ducklow and Mitchell 1979b). Mucus-associated bacteria generally differ from those present in the seawater, and can include *Sphingobacterium*, *Endozoicomonas*, *Tumebacillus* and *Ruegeria,* among others (Apprill et al. 2016; Glasl et al. 2016; Kooperman et al. 2007; Kvennefors et al. 2010; Sweet et al. 2011). The composition of the mucus microbiome changes with coral age and disturbance, highlighting their dynamic and responsive nature (Bent et al. 2021; Glasl et al. 2016). The mucus microbiome is thought to play a role in regulating coral health, through the production of antibiotic compounds (Ritchie 2006) and protection against bleaching and necrosis (Glasl et al. 2016). Studies of coral disease show that the mucus microbiome composition alters prior to lesion formation, suggesting that the mucus microbiome may be an early indicator of disease (Huntley et al. 2022).

Corals harbor microbes within their tissues, including within the epidermal and gastrodermal tissue layers (Ainsworth et al. 2006; Ainsworth and Hoegh-Goldberg 2009; Hernandez-Agreda et al. 2016; Lesser et al. 2004). Sequencing-based studies abound and offer insight into the identity of these coral-associated microbes. However, coral tissue-associated bacteria have been localized using fluorescent probes and microscopy in a limited number of cases, and I will highlight three specific tissue-associated bacteria in this chapter: nitrogen-fixing cyanobacteria, *Endoziocomonas* and "*Candidatus* Aquarickettsia rohweri". A key study that linked the identify, location and function of bacteria within corals was the discovery by Lesser et al. (2004) of nitrogen fixing cyanobacteria within the epithelial tissues of orange fluorescing corals. Follow-up work reported the rates of nitrogen fixation and fate of this new nitrogen into *Symbiodinaceae* cells (Lesser et al. 2007). Interestingly, the phylogenetic identity of the cyanobacteria remains unresolved, and there is an opportunity for further research on this relationship.

The Gammaproteobacteria *Endozoicomonas* is a common tissue associate of corals (Neave et al. 2017b). Further, *Endozoicomonas* phylotypes show host phylogenetic patterns (Pollock et al. 2018). *Endozoicomonas* were first discovered to form dense assemblages within the epidermis and gastrodermis of the common Pacific corals *Stylophora pistillata* and *Pocillopora meandrina* (Neave et al. 2016, 2017b), and similar aggregations have been further observed in diverse species, sometimes in association with other bacteria (Maire et al. 2023; Wada et al. 2022). *Endozoicomonas* are facultative symbionts which are also able to reside free-living within the water column (Weber et al. 2019). *Endozoicomonas* are hypothesized to play a beneficial role in coral nutrition, through sugar transport and utilization and protein secretion and also show evidence of plasticity which may enable adaptation to new conditions, and this was shown in the first metagenomic-assembled genome and single cell sequencing study of a coral-associated bacteria (Neave et al. 2017a). *Endozoicomonas* show evidence of host interaction, through expression of vitamin B1 and B6 synthesis genes and glycolytic processes, when exposed to the coral host, importantly demonstrating the first observations of coral-bacterial interchange (Pogoreutz et al. 2022). There is also some evidence for dimethylsulfoniopropionate (DMSP), an osmolyte produced by algal symbionts, use as a carbon source (Tandon et al. 2020), although this has not been shown in all *Endozoicomonas* genomes (Neave et al. 2017a). A recent study shows that heat-stressed *Endozoicomonas* can use cholesterol as a carbon source as well as a substrate to synthesize key progesterone and testosterone, potentially interacting with coral reproductive, oxidative regulation and other immune functions (Ochsenkuhm, Mohamed et al. 2023). *Endozoicomonas* are generally associated with healthy appearing, non-diseased corals (Huntley et al. 2022; Morrow et al. 2012; Neave et al. 2017b), suggestive of a beneficial role. Further phylogenetic research has described a new coral-associated Endozoicomonadaceae genus and species, highlighting what is likely a rich opportunity for further discovery within this family (Maire et al. 2024). However, while our knowledge of coral-associated *Endozoicomonas* interactions are growing (Pogoreutz et al. 2022), it is yet to be determined if *Endozoicomonas* is a beneficial symbiont of coral (Pogoreutz and Ziegler 2024).

A parasitic tissue-associated bacteria of corals is the alphaprotebacterium "*Candidatus* Aquarickettsia rohweri" (Klinges et al. 2019). This ubiquitous associate has a pervasive association with Caribbean *Acropora* corals, appearing in histological specimens since the 1970s (Peters 1984; Peters et al. 1983). Recent research has localized *A. rohweri* within coral epithelia (Klinges et al. 2019) and shown that abundances in *Acropora cervicornis* increase following prolonged nutrient exposure, which also coincides with decreased host growth and increased onset or progression of white band disease (Klinges et al. 2020; Shaver et al. 2017). Genomic analysis has shown that *A. rohweri* lacks sugar and amino acid synthesis genes, and possesses the ability to respond to a host (Klinges et al. 2019). *A. rohweri* is likely transmitted horizontally between corals (Baker et al. 2022). *A. rohweri* appears to be a parasite of Caribbean acropoids, but the nature of its association with other corals and cnidarians remains to be investigated (Klinges et al. 2019).

Corals also contain endolithic microorganisms, or microbes residing in the pore spaces within the skeletal matrix. Endolithic microbes have been shown to contain the largest reservoir of coral microbial diversity (Pollock et al. 2018), which is likely attributed to the unique and variable physiochemical characteristics influenced by the porous skeleton and overlying coral tissue layer. Indeed, light, oxygen, pH and nutrients differ by large degrees within this habitat, depending on the physical location, surrounding microbes and time of day (reviewed within Pernice et al. 2020). Prokaryotes are commonly associated with the endolithic algal and fungal skeletal bands, and include the filamentous marine cyanobacteria (Yamazaki et al. 2008) and nitrogen fixing, green sulfur bacteria (Yang et al. 2016, 2019), likely providing primary production and nitrogen benefits to the coral holobiont. Boring cyanobacteria (e.g., *Mastigocoleus testarum*) can also be associated with coral skeletons, and contribute to microerosion of the coral skeleton (Ramírez-Reinat and Garcia-Pichel 2012). The overall composition of the coral skeletal microbiome tracks host phylogeny more strongly compared to the mucus and tissue, suggesting that host-based traits drive skeletal microbiome diversification (Pollock et al. 2018). Overall, coral endoliths are understudied and their impact on the holobiont remains to be fully recognized.

3.5.2 Role of the Coral Microbiome in Contributing to Coral Health

There is significant interest in developing applied uses of the coral microbiome to diagnose and improve the health of corals. Indeed, microorganisms often serve as sensitive and rapid sensors of disturbance and change, due to their relatively rapid rates of growth and specific growth requirements (Ager et al. 2010; Atlas et al. 1991). Within corals, disturbance events have resulted in community changes within their microbiome, which could impact both microbiome and host functioning. For example, alterations in the composition, richness and abundance of the coral microbiome have been related to biological events (algal competition, reproduction, disease and other compromised states) and environmental changes (temperature, pH, nutrients, and light) (reviewed within Bourne and Webster 2013; Bourne et al. 2016). Understanding if and how the microbiome changes under these scenarios is necessary to understand the role of the microbiome in providing resilience and resistance to coral functioning.

Research suggests that coral microbiomes contribute to the heat tolerance of corals (Gilbert et al. 2012; Ziegler et al. 2017), and this has led to the idea that coral microbiomes may provide corals with resistance and resilience to thermal-stress conditions. The Beneficial Microorganisms for Corals (BMC) hypothesis (Peixoto et al. 2017) suggests that certain coral microbes play a beneficial role in coral health, and that probiotics may provide a means to assist in the acclimation and adaptation of corals to environmental conditions. Follow-up work on this hypothesis shows the promise for microbes to play a probiotic-type role in coral health (Rosado et al. 2019; Ushijima et al. 2023) (refer to Chap. 13).

3.5.3 Outlook and Future Research Needs for Coral-Associated Microbiology

Coral microbiomes are a critical area of research, which is partly motivated by the need to mitigate coral stress and improve their resilience (Voolstra et al. 2021, 2024). There is great opportunity in this field to move beyond descriptions of microbial taxa into specific host-microbe interactions and dynamics. I see two key opportunities to enhance coral microbiome research. One is the application of quantitative methods such as taxon-specific quantitative PCR or fluorescent in situ hybridization to better quantify and observe microorganisms in corals. Coral microbiomes are most commonly examined using 16S ribosomal RNA gene-based methods, applied by hundreds of studies over the past decade (reviewed within McDevitt-Irwin et al. 2017; Mohamed et al. 2023). While 16S-based analyses are semi-quantitative for many fields such as studies of marine picoplankton which utilize discrete volumes of seawater (e.g., Martin-Platero et al. 2018), this method appears to be far less quantitative for coral tissues. In most corals, the associated microbial cells are very low in biomass compared to the abundant and larger sized coral and Symbiodiniaceae, and this leads to challenges with the efficiency of DNA extraction as well as PCR amplifications (Silva et al. 2023; Weber et al. 2017). Examination of coral-associated microbial dynamics over time or treatment (such as probiotic additions) should be especially motivated to utilize quantitative or multiple methodological approaches (e.g., taxon-specific FISH) to confirm 16S rRNA gene-based community dynamics.

The second area of opportunity is towards understanding coral-microbial interactions. The three coral tissue-associated taxa described above, nitrogen fixing cyanobacteria, *Endozoicomonas* and *A. rohweri* represent our limited knowledge of coral-bacterial interactions, and even these are incomplete. While there are pieces of knowledge about other specific microbes interacting with corals, we need enhanced investigation into coral-microbial symbiosis including mechanisms of interaction (Mohamed et al. 2023; Pogoreutz et al. 2022). Further, with the increased focus on reef restoration and solutions to rebuild reefs, we cannot lose sight of the still deep need for fundamental knowledge about how corals interact with their microbial symbionts, and the mechanisms of how microbial probiotics enhance coral health under thermal stress. Enhanced funding, such as inclusion in the next phase of the Gordon and Betty Moore Foundation's 'Symbiosis in Aquatic Systems Initiative' could catalyze this need. Furthering collaborative opportunities between coral reef and symbiosis researchers in other fields could also help towards this goal.

3.6 Reef Fish Microbiomes

3.6.1 General Features

Reef fish are one of the most biodiverse communities on reefs, comprising 6000–8000 species (Reimchen 2002). Reef fish are mobile within the reef, with small and often cryptic species residing in reef crevices while others span larger areas of the reef. Reef fish diet varies by species, and includes herbivorous fish that ingest algae, corallivore fish that rely on coral and calcareous algae and carnivorous fish that feed on other organisms. The high diversity of reef fish paired with the differences in mobility and diet makes for a large range of potential microbial interactions. However, there is limited knowledge about the microbiomes of reef fish and the factors that influence microbiome development.

Microbial community composition and diversity in reef fish is structured according to body site. The skin of coral reef fish is coated in mucus and contains a diverse community of prokaryotes (~600 operational taxonomic units), with host phylogeny and diet (Chiarello et al. 2018) as well as reef habitat contributing to observed community differences (Pereira et al. 2023; Xavier et al. 2020). Despite their close proximity with the reef water, these skin-associated communities differ from the common seawater microorganisms and include Vibrionaceae, Alteromondales, Rhizobiales and Clostridiales (Chiarello et al. 2018) and specific genera *Pseudomonas*, *Ekhidna* and *Psychrobacter* (Pereira et al. 2023). The gills of fish, responsible for respiration and waste exchange, differ between juvenile and adult fish, including *Shewanella* and Endozoicomonaceae, with compositional differences related to host phylogeny and diet (Pratte et al. 2018).

More research has focused on fish intestinal microbiomes. The most well studied includes the relationship between herbivorous surgeonfish and *Epulopiscium* spp., an unusually large bacterium (~0.7 mm) that persists within the the gut (Clements et al. 1989; Fishelson et al. 1985; Grim et al. 2013). *Epulopiscium* possesses strategies for efficient nutrient and energy capture to support its large size (Sannino et al. 2023). Studies further describe relationships between host phylogeny and diet. Reef fish intestines are enriched in Brevinemataceae and *Epulupiscium* and diverse cyanobacteria which may be food-associated, compared to the gills (Pratte et al. 2018). A comparison of damselfish belonging to planktivorous (plankton diet) or algae-farming (algae diet, via farming for specific alga) guilds found that the algae farming fish had higher alpha diversity and a more diverse core microbiome (35 ASVs) compared to the planktivorous species (7 ASVs) (Kavazos et al. 2022). Further, the intestines of algae farming damselfish were enriched in Pasteurellaceae and planktivorous intestines Vibrionaceae (Kavazos et al. 2022). Location sampled within the gastrointestinal tract (stomach vs. anterior intestinal regions) also influences the microbes residing there, likely due to the differing physiochemical and nutrient properties (Kavazos et al. 2022). Further, residency of the fish influences the gut microbiome, with pre-settlement damselfish and cardinalfish enriched in Endozoicomonaceae, Shewanellaceae and Fusobacteriaceae and settled fish gut comprised of Vibrionaceae and Pasteurellaceae (Parris et al. 2016).

3.6.2 Fish Microbiome Dynamics and Coral Reef Ecosystem Health

There are connections between aspects of the fish microbiome and the health and dynamics of the coral reef ecosystem. Specific disruptions lead to microbial alterations. For example, fish gill microbiomes can be disrupted by high sediment loads, with enrichment of gill-associated microbes related to pathogens (Hess et al. 2015). Further, nutrient enrichment influences the intestinal microbiome (both hindgut and foregut) of damselfish (Degregori et al. 2021). On a larger scale, the gut microbiomes of butterflyfish residing on degraded reefs have signatures of a lower coral-associated diet, including lower relative abundances of *Endozoicomonas* and higher diversity of anaerobic fermentative bacteria (Clever et al. 2022). Further, butterflyfish are hypothesized to serve as major coral disease vectors, due to their feeding on stony corals and prominence on overfished reefs (Raymundo et al. 2009).

In contrast to a benthic organism such as a coral, the mobility of reef fish provides them with the ability to shape and be shaped by various parameters and features of the reef, and this may influence microbial distribution and delivery on reefs. Fish feces are hypothesized as a mechanistic link between the pelagic and coral reef food webs, due to the nutrient and carbon transfer occurring via feces (Meyer and Schultz 1985). Fish feces are also sources of food for some fish species (Bailey and Robertson 1982). Skin microbiomes of goby fish associated with cleaning stations, areas of the reef fish visit for parasite removal, differ in alpha and beta diversity from those in control plots of the same reefs, suggesting that these cleaning stations may serve as areas of microbial exchange on reefs (Pereira et al. 2023).

3.6.3 Outlook and Future Research Needs for Reef Fish-Associated Microbiology

Given the high diversity of reef fish and its recent exploration, reef fish microbiology is still largely in an observational phase. As more studies become available, there is a need for meta-analysis, or a comparison of data and trends across studies and species, similar to compilations in other reef environments (Huggett and Apprill 2019; Rosales et al. 2023; Thomas et al. 2016a). There is also an opportunity to integrate fish microbiology into the larger ecosystem, by further examining how fish presence/diversity relates to coral disease and the coral microbiome, for example (Raymundo et al. 2009). Attention towards developing model fish organisms for study will pave the way for more mechanistic studies, such as drawing connections between the fish microbiome and social behavior (Soares et al. 2019) or ecosystem health (Clever et al. 2022; Raymundo et al. 2009). Finally, there is an opportunity for applied research in this area, specifically examining if fish can serve as vectors for beneficial microorganisms, which could be particularly useful for reef restoration.

Key Takeaways

1. Coral reef microbiology has been an active field of research for nearly 70 years, with phases including early

observations, technology-enhanced discoveries, and a growingly important era examining the role of microorganisms in reef health, thermal resistance, and disease resilience.

2. Coral reef microorganisms are numerically abundant and diverse, residing in reef water and sediments and in association with reef surfaces and organisms.
3. Reef water is dominated by cyanobacteria (*Prochlorococcus* and *Synechococcus*) and oligotrophs (SAR11, SAR86), and these major community members as well as associated copiotrophs are emerging as diagnostic tools for environmental and reef-associated conditions.
4. Coral reef sediment microorganisms play roles in nutrient transformations; their role in providing novel antibiotic or pathogen traits to reefs requires additional research.
5. Coral-associated microorganisms vary by coral compartment (ecosphere, mucus, tissue and skeleton), are phylogenetically diverse, and show promise for providing beneficial traits to corals. Specific coral-microbial relationships and interactions is a critical area of future research.
6. Microorganisms associated with reef fish vary according to host phylogeny, anatomy, and diet; they may be involved with or reflect larger reef ecosystem processes and/or health.

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Coral Skeleton Dwelling Endolithic Algae: *Ostreobium* and Its Biology

4

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Abstract

The coral skeleton is home to a highly diverse microeukaryotic and prokaryotic community, including algae from the genus *Ostreobium:* siphonous green algae living inside the calcium carbonate coral skeleton and visible as conspicuous green bands beneath the coral tissue. This alga's broader potential functional role is a contemporary area of focus in coral reef ecology. This chapter summarises the current knowledge about *Ostreobium* biodiversity, its intriguing photobiology, microbiome, and functional role in the coral holobiont, and highlights some knowledge gaps related to the complex biology of this alga.

Keywords

Ostreobium · Endolith · Siphonous green algae
Low-light photosynthesis · Ostreobium-microbiome

4.1 Introduction: Exploring Hidden Worlds

Coral microbiomes are known to be compartmentalised, with the mucus, tissue and skeleton harbouring highly distinct microbiomes between each other and compared to the surrounding seawater (Sweet et al. 2011; van Oppen and Blackall 2019). Work done over the last few decades has led to a detailed understanding of the coral mucus and tissue microbiomes (Bourne and Webster 2013; Hernandez-Agreda et al. 2017; van Oppen and Blackall 2019), but coral skeleton microbiomes have only recently come into focus (Ricci et al. 2019; Pernice et al. 2020).

Endolithic environments within solid substrates, both terrestrial and marine, provide a crucial refuge for a variety of microbial life, offering protection from environmental (e.g., extreme solar radiation and desiccation) and supplying essential nutrients and moisture. These microorganisms inhabit the mineralized skeletons of marine animals, including corals, and are overall dominated by cyanobacteria, fungi, and green algae, such as *Ostreobium* spp. (Pernice et al. 2020; Ricci et al. 2019). Early molecular studies revealed more details of the highly diverse microeukaryotic and prokaryotic communities associated with the coral skeleton (Marcelino and Verbruggen 2016; Yang et al. 2016; Marcelino et al. 2018; Ricci et al. 2021). The microbial community is structured across the steep physiochemical gradients (light, oxygen and pH) of the coral skeleton, with upper tranches dominated by algae *Ostreobium*, cyanobacteria, *Myxococcales,* and the deeper skeleton, which arguably offers a more stable environment, dominated by anoxygenic phototrophic and heterotrophic bacteria (Kühl et al. 2008; Ricci et al. 2023). Metagenomics and nanoscale secondary ion mass spectrometry are starting to shed light on the functional potential of the coral skeleton microbiome (Cai et al. 2017; Yang et al. 2019; Tandon et al. 2022; Cárdenas et al. 2022). Some of the proposed vital roles of the endolithic microorganisms for coral health include nutrient cycling and transferring metabolites that may help corals withstand thermal stress (Cárdenas et al. 2022; Yang et al. 2019; Fine and Loya 2002). On the contrary, these microorganisms also have the ability to dissolve calcium carbonate, which can weaken coral skeletons and contribute to reef erosion (Tribollet et al. 2006; Tribollet et al. 2019; Fordyce et al. 2020; Pernice et al. 2020).

This chapter focuses on the microboring siphonous endolithic algae *Ostreobium*. It summarises current knowledge of its biodiversity, genomics, microbiome, adaptation to low light environment, and potential roles in the coral reef ecosystem.

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4.2 Biodiversity, Evolution, and Genomics

Ostreobium is a member of the Bryopsidales, a group of siphonous green algae (Verbruggen et al. 2009, 2017). It forms microscopic galleries in highly diverse marine calcium carbonate structures and occurs in temperate and tropical regions and across a broad bathymetric range (Odum and Odum 1955; Littler et al. 1985). Currently, there are five *Ostreobium* species formally recognised; the type species *O. quekettii* and four others differentiated based on morphological traits: *O. constrictum*, *O. duerdenii*, *O. okamurae*, and *O. reineckii* (Tandon et al. 2023). The type species *O. quekettii* was first described living in commercial oyster shells but limited information is available about the ecological niche(s) of other species because nearly all *Ostreobium* records have used the species name *O. quekettii*. Environmental sequencing of marker genes such as *tuf*A, *rbcL,* UPA and 16S rRNA have identified tremendous genetic diversity of *Ostreobium* in scleractinian corals and other carbonate substrates, providing clear evidence that the species diversity of the genus is underestimated (Marcelino and Verbruggen 2016; Gutner-Hoch and Fine 2011; del Campo et al. 2016; Gonzalez-Zapata et al. 2018), a finding that has strong support from culture-based studies as well (Sauvage et al. 2016; Massé et al. 2020; Pasella et al. 2022).

The recurrent mass bleaching of coral reefs, including four global coral bleaching events, have led to a significant push to identify the functional contributions of coral holobiont members as a step towards mitigating the effects of climate change on coral reefs through microbiome stewardship (Santoro et al. 2021; Peixoto et al. 2017; van Oppen et al. 2015; Peixoto et al. 2022). These efforts have increased the cultured isolates of *Ostreobium* available from culture collections (RBCell collection, Paris France; the Australian National Algal Culture Collection, ANACC, Hobart, Australia; Culture Collection of Algae at the University of Göttingen, SAG, Germany). Further, a range of genomic resources have been generated, including the nuclear genome of strain SAG 6.99 (Iha et al. 2021), chloroplast genomes of 14 strains (Pasella et al. 2022; Alesmail et al. 2023), and one mitochondrial genome (Repetti et al. 2020). These resources have shed light on the evolutionary history of *Ostreobium* and provided an initial set of insights into its adaptations to an endolithic lifestyle, including an expansion of genes related to photosynthesis and calcium transport reflecting its ability to photosynthesise in near-infrared light and its burrowing capabilities (Iha et al. 2021).

4.3 *Ostreobium* Photobiology

Light energy is essential for photosynthesis, and most algae capture light in the photosynthetically active radiation spectrum (PAR; from 400 nm up to 680 nm). As an endolithic organism, *Ostreobium* is specialised to living in very low light in healthy coral (reviewed in (Tandon et al. 2023)). Algal adaptations to extreme low-light habitats are generally linked to an increased size of the light-harvesting complexes (LHCs) to increase the amount of light absorption (Negi et al. 2020). In *Ostreobium*, LHCs contain approximately 340 chlorophyll (Chl) molecules (including both Chl *a* and Chl *b*) per Photosystem II reaction centre (PSII RC), which is significantly more than higher plants (~240 chlorophyll molecules per PSII RC) (Koehne et al. 1999), and *Ostreobium*'s light compensation point (where photosynthesis equals respiration) is low, around 10–50 μmol photons m^{-2} s^{-1} (Schlichter et al. 1997). In addition to these LHC adaptations, *Ostreobium* has experienced some genomic changes that have been linked to its low-light habitat, including a streamlined chloroplast genome, reduced rates of molecular evolution, and loss of the *cemA* gene encoding for chloroplast envelope membrane protein involved in high-light tolerance (Marcelino et al. 2016).

In the coral skeleton, probably the most common niche where *Ostreobium* has been reported, light availability to *Ostreobium* is significantly lowered by the zooxanthellae in the coral tissue (Wijgerde et al. 2014). These zooxanthellae use most of the PAR, leaving mainly far-red light for *Ostreobium* (Magnusson et al. 2007). Interestingly, no specific pigments for far-red light absorption are found in *Ostreobium*, contrary to cyanobacteria which are known to harbour chlorophylls *d* and *f* (Allakhverdiev et al. 2016). Rather, *Ostreobium*'s adaptation to far-red light is linked to a shift in pigment ratios between chlorophylls *a* and *b* (Wilhelm and Jakob 2006). Early studies have demonstrated that *Ostreobium* has different LHCs in the PSII, and specifically, the *Lhca*1 was the most commonly found protein during exposure to monochromatic far-red light (Koehne et al. 1999). The *Ostreobium* nuclear genome has shed light on adaptations at the protein level changes that could affect the light absorption spectra (Iha et al. 2021). The A5 site of the *Lhca1* gene shows a substitution of histidine with asparagine, which in *Arabidopsis thaliana* mutants results in a red-shifted absorption spectrum (Morosinotto et al. 2003).

While *Ostreobium* is a low-light specialist, it is known to bloom after coral bleaching due to an increased irradiance in the coral skeleton. Increased light is a double-edged sword: on the one hand it provides more energy helping algae to grow faster, but at the same time it can compromise the activity of the photosystems due to increased production of reactive oxygen species (Rezayian et al. 2019). Algae can deal with high light by limiting the damage to the PS (photoprotection mechanisms) or quickly replacing the damaged proteins (photo repair mechanisms).

Non-photochemical quenching (NPQ) is the most prevalent and efficient type of photoprotection mechanisms in algae (Ruban 2016). However, the *Ostreobium* nuclear genome lacks the *PsbS* gene involved in the faster energy-type (qE) NPQ (Iha et al. 2021) and laboratory experiments have reported that NPQ does not appear to be a predominant mechanism in *Ostreobium* (Pasella et al. 2023*)*. The xanthophyll cycle (XC) is another type of NPQ mechanism that appears to be absent in *Ostreobium*, and more broadly in the Bryopsidales (Iha et al. 2021). The siphonous structure of *Ostreobium* may also play a role in the photoprotection as it may enable chloroplast movement as previously described for other genera in the Bryopsidales (Giovagnetti et al. 2018). However, whether this happens in *Ostreobium* is yet to be explored.

Photorepair mechanisms relate to the replacement of the D1 protein responsible for binding the primary electrons donors and acceptors in the PSII (Theis and Schroda 2016). Ongoing work indicates a high repair rate of the D1 protein (Pasella et al. 2023), yet this is a very energy-demanding mechanism and ineffective as a long-term solution. Our understanding of how *Ostreobium* handles high-light energy will require additional studies to better interpret this alga's biology in the coral reef ecosystem.

4.4 The *Ostreobium* Microbiome

For the coral holobiont to work effectively, symbionts should interact with each other, including *Ostreobium* and the microbial communities residing in the coral skeleton. Recent work has started to characterise the microbial diversity of coral-associated algae (Maire et al. 2021; Pushpakumara et al. 2023b; Massé et al. 2023). The microbial community composition of cultured representatives from different *Ostreobium* clades was described in detail (Pushpakumara et al. 2023b) along with changes in microbial community composition in different salinities (Massé et al. 2023). These studies identified that *Ostreobium* associates with diverse bacterial groups, many of which are known to occur in the coral skeleton, including, *Kiloniellaceae*, *Rhizobiales*, *Candidatus Amoebophilus,* members of *Rhodobacteraceae, Cyclobacteriaceae,* and *Rhizobiaceae* (Pushpakumara et al. 2023b; Massé et al. 2023). The associations were genotype-specific, with a small core microbiome and high inter- and intra-species variability, as shown in other siphonous *Bryopsidales* (Aires et al. 2015) and green microalgae (Piampiano et al. 2019). Further, phylosymbiotic signatures are present in the core-bacterial taxa of *Ostreobium*, making it the first alga-bacterial system to show this pattern (Pushpakumara et al. 2023b).

Ostreobium microbiome studies have revealed an association with methylotrophs, for instance the genera *Methyloceanibacter* and *Leisingera* had consistent and significant abundance (Pushpakumara et al. 2023b). This observation is important as *Symbiodineaceae* were recently also shown to harbour a methylotroph, *Methylobacterium*, as an intracellular member of its core microbiome (Maire et al. 2021). The association of algal symbionts of corals with methylotrophs points to a putatively tight relationship, similar to that observed between terrestrial plants and methylotrophs, where these bacteria promote plant growth by stimulating plant growth hormones and provide support for nutrient uptake (Trotsenko et al. 2001; Vorholt 2012). These bacteria could potentially play a role in the metabolism of C1 compounds in the holobiont (Pushpakumara et al. 2023b).

Using Catalyzed Reporter Deposition Fluorescence In Situ Hybridization (CARD-FISH), *Candidatus Amoebophilus* and *Rickettsiales* were flagged as potential intracellular bacteria of *Ostreobium* (Massé et al. 2023). Members of *Candidatus Amoebophilus* lineage are obligate intracellular symbionts first discovered in marine amoebae (Kawafune et al. 2012) and are abundant in the skeleton of many coral species (Ricci et al. 2022). Although a metagenome-assembled genome (MAG) has been recovered for *Candidatus Amoebophilus* from *Ostreobium* cultures (Pushpakumara et al. 2023a), sharing genomic features with *Candidatus Amoebophilus asiaticus*, including a highly reduced genome, enrichment of eukaryotic-like repeat proteins, Insertional Sequence elements, and protein secretion systems that may facilitate symbiosis with the host (Schmitz-Esser et al. 2010; Selberherr et al. 2022). Yet this bacteria's functional roles in the coral holobiont require further study.

Rickettsiales are obligate intracellular bacteria, identified and sequenced from unicellular green algae *Carteria* and two Bryopsidales (Hollants et al. 2013). Further, intracellular *Rickettsiales* have been detected in coral tissue (Klinges et al. 2019) and in Symbiodinaceae isolated from *Acropora tenius* and *Porites lobata* (Maire et al. 2021). Identification of similar taxa in the two dominant algal symbionts of corals suggests potential synergies between *Rickettsiales* and coral-associated algal symbionts. However, our knowledge about the functional potential of these algal symbionts is in its infancy. We have assembled a compendium of bacterial MAGs recovered from *Ostreobium* cultures, depicting high potential for a multitude of functions and algal-association (Pushpakumara et al. 2023a), but these will require detailed molecular and functional studies to characterise functions and interactions.

4.5 *Ostreobium* in the Healthy and Bleached Coral Holobiont

Reef-building corals depend on their tissue-dwelling endosymbionts (Symbiodineaceae) for energy acquisition(LaJeunesse et al. 2018). Rising seawater temperatures can trigger the breakdown of this obligate symbiosis, causing coral bleaching, and death if the bleaching is not reversed (Hoegh-Guldberg 1999). During bleaching events, corals are almost devoid of Symbiodineaceae, but *Ostreobium* is now receiving more light in the skeleton, leading to increased photosynthetic activity, causing it to bloom. This increased photosynthetic activity suggests *Ostreobium* may become an alternate energy source for the coral. Using Carbon-14-labelled bicarbonates, Schlichter et al. (1997) provided the first evidence that *Ostreobium* photosynthate was translocated to aposymbiotic *Tubastraea micranthus*, and incorporated into the tissue lipids. Since then, other studies have also shown the incorporation of *Ostreobium*-produced photosynthates into the coral tissue of bleached and healthy Symbiodiniaceae-harbouring corals (Fine and Loya 2002; Sangsawang et al. 2017; Cárdenas et al. 2022), however, the level of contribution these photosynthates make to the coral's energy budget or how *Ostreobium* blooms affect the holobiont at large, remain elusive. Besides providing photosynthates to bleached corals, *Ostreobium* has been hypothesised to have other beneficial roles, including providing partial protection from high-light stress and promoting coral recovery after coral bleaching (Yamazaki et al. 2008; Galindo-Martínez et al. 2022; Fine and Loya 2002; Rodríguez-Román et al. 2006). Negative effects include the calcium carbonate dissolution that *Ostreobium* causes, which would affect the skeletal integrity of the coral. *Ostreobium* is the principal microbial agent of tropical reef decalcification, eroding ca. 1 kg $CaCO_3$ per square meter of reef surface per year (Tribollet 2008), but so far, relatively little work has been done on the impacts of its bioeroding function in live corals (Reyes-Nivia et al. 2013).

4.6 Conclusion

Despite advances in our understanding of *Ostreobium* biology, many questions remain about the complex relationships between *Ostreobium* and other coral holobiont members, some of which require urgent answers to better understand and model the coral reef ecosystem, including how this endolithic alga affects the fitness of corals across their life span and whether this may change in warmer, more acidic oceans.

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Viruses

5

Bailey Wallace, Natascha Varona, and Cynthia Silveira

Abstract

Viruses are ubiquitous members of the coral holobiont and the coral reef ecosystem. They infect coral cells, endosymbionts, prokaryotes, and all reef inhabitants, contributing to their physiology and ecological relationships. Here, we review the current knowledge on the diversity and functions of viruses in corals and coral reefs. We describe the viral groups that are found in most coral viromes and the shifts in the viral community composition associated with environmental stress and disease. Selective lysis of host cells by viruses modulate microbiome composition, and virus-mediated genetic exchange increases genomic flexibility of hosts. Through these mechanisms, viruses may contribute to acclimation and adaptation of coral holobionts, including resilience against temperature stress and protection against pathogens. We also explore how coral reef viruses modulate reef-scale processes such as organic matter turnover, biomass transfers, and biogeochemical cycles. Current data indicate that predation pressure exerts an important control of bacterial biomass and the detrimental microbialization of coral reefs. Finally, we identify challenges in virus research and future directions toward a mechanistic understanding of the role of viruses in coral reefs.

Keywords

Herpesvirus · NCLDV · dinoRNAV · Bacteriophage · Lysis · Lysogeny · Lateral gene transfer

5.1 Introduction

With an estimated 10^{31} particles across all ecosystems, viruses are the most abundant biological entity in our world. They possess a common structural framework consisting of genetic material, either in the form of DNA or RNA, and a capsid and/or envelope that protects the genetic material and mediates host infection. The replication of viruses is contingent upon the availability of a host cell, which can belong to any domain of life. While viruses are primarily viewed as agents of disease, they also promote genetic diversification of their hosts and modulate community structure, playing a crucial role in host ecology and evolution. Because viral infection can culminate in host death, viruses exert selective pressure on cells to evolve countermeasures to evade infection. In response, viruses evolve to evade the defensive strategies of their hosts. This co-evolutionary process is shaped by the complex ecological interactions of viruses and hosts with their environments.

The study of viruses in corals and coral reefs is a relatively new field of investigation, with the earliest works identifying viral particles in corals, anemones, and sponges dating from the late 1990s and early 2000s (Scanlan and Wilson 1999; Wilson et al. 2001; Cervino et al. 2004). These early studies of viruses in corals focused primarily on their potential to cause disease under environmental stress (Wilson et al. 2005; Davy et al. 2006). The emergence of genomic data showed the enormous diversity of coral-associated viruses in both healthy and diseased states, with viruses infecting the coral animal cells, the symbiotic dinoflagellates, and all other members of the holobiont, and suggesting that viruses contribute to key holobiont functions (Marhaver et al. 2008; Vega Thurber et al. 2008; Wood-Charlson et al. 2015; Soffer et al. 2015).

Viruses modulate the microbial community composition and function associated with different animals, and mediate colonization by foreign microbes (Barr et al. 2013; Hsu et al. 2019). These widely consistent interactions suggest that

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viruses may play similarly important roles in the coral holobiont. Given the importance of microbes for coral function, adaptation, and response to stress, it is predicted that the viruses infecting these microbes also play an important role in these processes, as suggested by genomic data. However, there is still little experimental evidence for these functions in corals, and the mechanistic and causal roles of viruses in coral health and disease are yet to be described. This chapter reviews the current knowledge on the diversity of viruses in coral holobionts, describes the potential roles of these viruses, mostly inferred from metagenomic studies, and discusses some of the challenges and future directions for a mechanistic understanding of the importance of viruses for coral health and adaptation.

5.2 The Coral Virome

Over 60 different viral families inhabit the coral holobiont, encompassing a diversity of morphologies, nucleic acid compositions, and hosts (Fig. 5.1) (Davy and Patten 2007; Marhaver et al. 2008; Wood-Charlson et al. 2015; Vega Thurber et al. 2017; Weynberg et al. 2017; Cárdenas et al. 2020). Viruses lack a universally conserved marker gene like the 16S ribosomal gene used for taxonomic classification of Bacteria and Archaea (Koonin et al. 2020). Therefore, we rely on whole genome similarity to known viruses to identify and classify viral sequences (these challenges are further discussed in the section "Challenges and Future Directions" below). Despite these limitations, we have improved our knowledge on the diversity of viruses in corals using shotgun metagenomics in the past decade. From this work, a "core coral virome" has been described, composed of 9 to 12 of the 264 viral families described by the International Committee for Taxonomy of Viruses, ICTV (Vega Thurber et al. 2017; Walker et al. 2022). This core virome was defined as viruses found in 90% or more of 12 coral species included in a meta-analysis of 14 studies (Wood-Charlson et al. 2015; Vega Thurber et al. 2017). The abundance and prevalence of these viruses can vary depending on coral species, geographic location, and physiological state of corals.

Double-stranded DNA (dsDNA) group I viruses are the most numerous and well-described within the core virome, infecting both eukaryotic and bacterial members of the holobiont. Among dsDNA viruses that infect eukaryotes, Nucleocytoplasmic Large DNA Viruses (NCLDVs) are ubiquitous. NCLDVs belonging to the families *Phycodnaviridae* and *Mimiviridae* are present across all corals studied to date and are inferred, based on microscopy and genomic data, to infect the coral host and *Symbiodiniaceae* (Correa et al. 2013, 2016; Levin et al. 2017; Weynberg et al. 2017; Cárdenas et al. 2020). Also present in the majority of corals are NCLDVs belonging to the families *Poxviridae, Iridoviridae*, and *Ascoviridae* (Wood-Charlson et al. 2015; Vega Thurber et al. 2017). Known *Poxviridae* typically infects vertebrates and arthropods, *Iridoviridae* infects phytoplankton and fish, and *Ascoviridae* primarily infects invertebrates. However, identifying their hosts within the coral holobiont has been challenging (Wood-Charlson et al. 2015; Vega Thurber et al. 2017).

The *Herpesvirales* order (*Alloherpesviridae*, *Herpesviridae*, and *Malacoherpesviridae* families) is another group of dsDNA viruses commonly associated with corals, albeit with highly variable abundance. A number of studies have found these herpes-like viruses to be among the most abundant dsDNA viruses in coral metagenomes (Marhaver et al. 2008; Vega Thurber et al. 2008; Vega Thurber and Correa 2011; Soffer et al. 2014; Correa et al. 2016), while others have found herpes-like sequences to represent less than 0.1% of eukaryotic viruses in the samples (Wood-Charlson et al. 2015; Weynberg et al. 2017). These differences may be a result of real biological differences between coral species, geographic locations, physiological states, or may arise due to the different analytical methods (Wood-Charlson et al. 2015; Weynberg et al. 2017). For example, a metagenomic study of seven species in the Great Barrier Reef observed low herpesvirus abundances, while a study of Acroporids in the same geographical region found, through transmission electron microscopy and metagenomics, a high abundance of this viral group (Correa et al. 2016; Weynberg et al. 2017). It is important to emphasize that though these herpes-like viruses are morphologically similar to known herpesviruses, sequence data suggests that they share relatively low sequence similarity to the reference genomes of herpesviruses infecting vertebrates (Wood-Charlson et al. 2015).

The most abundant and diverse viruses detected in corals are the dsDNA viruses that infect bacteria, known as bacteriophages or simply phages. Tailed bacteriophages (class *Caudoviricetes*), which includes the groups previously known as families *Myoviridae*, *Podoviridae* and *Siphoviridae,* are omnipresent in corals and represent the most abundant viruses within the holobiont (Laffy et al. 2016; Correa et al. 2016; Cárdenas et al. 2020). These families were historically characterized by the tail morphology of the viral particle, and genome membership within these groups was defined by sequence similarity to cultivated members. However, this classification scheme does not fully capture the evolutionary histories and relatedness of viral genomes, which revealed viruses of distinct morphologies but closely related genomes and vice-versa, and the International Committee for Taxonomy of Viruses (ICTV) has ended the recognition of these morphological groups as viral families (Simmonds et al. 2017; Gorbalenya 2018; Koonin et al. 2020; Walker et al. 2022). A new meta-analysis of viral genomes from corals under the light of a taxonomic framework based on evolutionary genomics shows that the tailed bacteriophage families *Kyanoviridae* and *Autographiviridae* are the most abundant, and that most tailed phages were predicted to infect *Alphaproteobacteria* (Wallace et al. 2024)

Single-stranded DNA (ssDNA) viruses of group II are also common members of the coral virome. For example, eukaryote-infecting ssDNA *Circoviridae* have been found in higher abundance in bleached and white plague diseased *Orbicella annularis* tissues (Soffer et al. 2014). However, methodological biases have made it challenging to accurately quantify the abundance of these viruses in coral samples: DNA amplification methods commonly used in early metagenomic studies used the Phi29 DNA polymerase, which has preference for circular ssDNA and may inflate their detection, while transcriptomes may not contain ssDNA virus annotations because of the enrichment for polyadenylated RNA (Wegley et al. 2007; Littman et al. 2011; Vega Thurber et al. 2017).

The least understood members of the core virome are retrovirus group IV. True retroviruses have been found in a number of studies (Correa et al. 2013; Rose et al. 2016; Correa et al. 2016), including the families *Retroviridae* (ssRNA retro-transcribing) and *Caulimoviridae* (dsDNA retro-transcribing). Retroviruses may not be well represented across the majority of samples as they are often indistinguishable from retroelements in the host genome and thus removed from annotations altogether (Vega Thurber et al. 2017). While *Retroviridae* are inferred to infect the coral host cells, the hosts of *Caulimoviridae*, which typically infects plants, are uncertain (Cárdenas et al. 2020).

5.2.1 Viruses of Eukaryotes

Transmission Electron Microscopy (TEM) demonstrates the presence of viruses in all layers of healthy and diseased coral tissues including gastrodermal, epidermal, and mesogleal layers (Fig. 5.1) (Patten et al. 2008; Bettarel et al. 2013). Viruses are likewise found to infect the extensively studied algal symbionts of corals, the *Symbiodiniaceae* (Wilson et al.

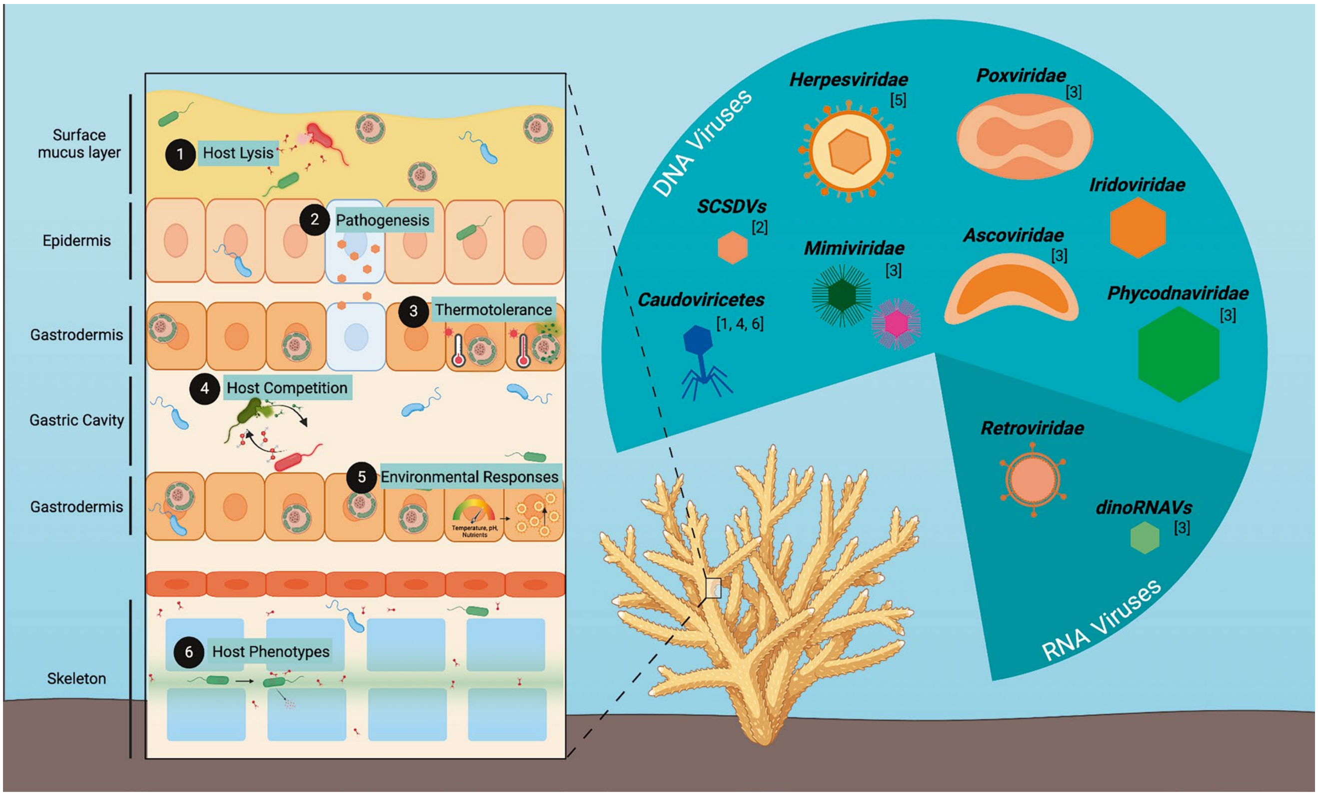

Fig. 5.1 Diversity, distribution, and potential functions of viruses in the coral holobiont. (1) Phages enriched in coral mucus may contribute to the lytic removal of bacterial pathogens. (2) SCSDVs are enriched in white plague-diseased coral tissues while absent in healthy corals, suggesting a potential role of these viruses in the pathogenesis of white plague disease. (3) +ssRNAVs, NCLDVs, and unclassified viruses are implicated in the thermotolerance and bleaching response of *Symbiodiniaceae* and some have been found to lyse cultured *Symbiodiniaceae* isolates when exposed to thermal stress. (4) Integrated prophages may be exploited by competing bacterial strains, playing a role in the colonization competition of bacteria. (5) Herpes-like viral particles and sequences increase in abundance with environmental stressors that cause coral bleaching such as pH, temperature, and nutrient availability. (6) Integrated prophages may modulate the metabolism and virulence of their bacterial hosts. Right: 10 of the most prevalent viral groups in corals colored by their inferred host (Blue: Bacteria; Orange: Coral; Green: *Symbiodiniaceae* or Endolithic Algae; Pink: Protists). Figure created with BioRender.com

2005; Davy et al. 2006; Lohr et al. 2007). These discoveries, in conjunction with metagenomics (Wegley et al. 2007; Marhaver et al. 2008; Vega Thurber et al. 2008), have unveiled a wide range of viral diversity within the eukaryotic components of the coral holobiont. The compartments of the coral animal (e.g. surface mucus layer, tissue, and skeleton) and the *Symbiodiniaceae* phycosphere *in hospite* can create microenvironments with distinct microbial communities (Sweet et al. 2011; Hill et al. 2024). These eukaryotic compartments may likewise select for unique viral associations with unique functions.

Viral propagation and infection are influenced by a number of abiotic environmental triggers such as ultraviolet radiation (Jacquet and Bratbak 2003), nutrient availability (Scanlan and Wilson 1999), pH (Baumann et al. 2016), and temperature (Edgar and Lielausis 1964). These same triggers are implicated in coral bleaching and diseases across coral reefs worldwide. Early studies on coral holobiont viruses observed that these abiotic stressors can induce shifts in viral community composition and abundance within coral tissues (Wilson et al. 2005; Davy et al. 2006; Vega Thurber et al. 2008; Correa et al. 2013) and recent research further supports the association between eukaryote-infecting viruses and coral diseases (Table 5.1). Though these associations may be expected, it remains difficult to distinguish between cause and effect.

5.2.2 Eukaryotic Viruses in Coral Bleaching

Coral bleaching is the loss of the symbiotic algae, *Symbiodiniaceae*, from coral tissues, turning the tissue colorless so that the underlying white skeleton becomes apparent. This loss usually occurs in response to an environmental stressor, such as temperature increases or high solar irradiance (Brown 1997; Voolstra et al. 2021). Herpes-like viruses in the coral gastroderm have been observed to increase in abundance in response to abiotic stressors associated with bleaching, such as reduced pH, elevated nutrients, and increased temperature (Vega Thurber et al. 2008; Correa et al. 2016). In a tank study exploring the effects of bleaching on *Pavona danai*, *Acropora formosa*, *Stylophora pistillata*, *Zoanthus sp.*, and their symbiotic *Symbiodiniaceae*, heat-shocked corals produced virus-like particles (VLPs) with distinct morphologies such as rounded, rod-shaped, droplet-shaped, and filaments (Davy et al. 2006). The most common VLPs observed were tail-less, hexagonal, and measured approximately 40–50 nm. Significantly, exposure of non-stressed *Symbiodiniaceae* isolates to VLPs from heat-shocked *Symbiodiniaceae* isolates resulted in cell lysis (Davy et al. 2006). Likewise, a proteomic study observed a remarkable 114-fold increase in the expression of a viral replication proteins during a 31 °C heat-shock treatment of *Symbiodiniaceae*-enriched tissue (Weston et al. 2012) and a thermosensitive *Symbiodiniaceae* exhibited decreased expression of +ssRNAV transcripts and increased expression of anti-viral transcripts at higher temperatures (Levin et al. 2017). In a study of *Acropora* sp. and their *Symbiodiniaceae* during a viral outbreak associated with a local bleaching event, TEM revealed an abundance of viruses with morphologies similar to retroviruses, herpesviruses, and NCLDVs, which was validated by sequence data (Correa et al. 2016). Together, these findings suggest that viruses infecting *Symbiodiniaceae* are influenced by heat stress and may contribute to bleaching.

Recent studies investigating viruses in the *Symbiodiniaceae* of heat-stressed *Porites sp.* both in situ and ex situ also propose a role of ssRNA viruses in thermal tolerance. Corals exposed to thermal stress exhibited an enrichment of positive-sense single-stranded RNA viruses (dinoRNAVs), indicating a shift from persistent to productive infections of *Symbiodiniaceae* (Grupstra et al. 2022). This work also identified a subset of 17 viral aminotypes to be significantly associated with heat-treated fragments. In *Porites lobata* sampled over distinct spatial and temporal gradients, 90% of the corals had detectable dinoRNAV infections (Howe-Kerr et al. 2023). The composition and richness of viral major capsid protein amino types were influenced by the reef zone, with the warmest reef zone containing the highest aminotype richness. While these studies suggest that viruses are correlated with bleaching, it is possible that viruses are not causing bleaching and instead responding to bleaching (which may exacerbate it). Coral bleaching could compromise the host's immune response, making them more susceptible to disease and infection. Environmental stressors other than temperature, such as ultraviolet light could also increase viral load through the induction of productive infections of *Symbiodiniaceae* (Lohr et al. 2007). Further research is needed to decouple the causal relationship between viral infection and bleaching.

5.2.3 Eukaryotic Viruses in Coral Diseases

White plague (WP) diseases of coral have contributed to the decline of tropical reefs worldwide, yet their etiology remains unclear. Some studies have attributed cases of white plague to bacterial pathogens (Denner et al. 2003; Thompson et al. 2006) or a consortium of microbes (Garcia et al. 2013), while other work has noted viral associations with diseased corals (Barash et al. 2005; Soffer et al. 2014; Daniels et al. 2015). It is important to note that putative viral roles in the etiology of white plague are yet to be mechanistically tested or satisfy with Koch's postulates. Barash et al., 2005 identified a virus-sized "filterable factor" (smaller than 0.2 μm)

Table 5.1 Viral associations with coral diseases

Disease/status	Host(s)	Evidence	Method	Reference
Bleaching	*Pavona danai, Acropora formosa, Stylophora pistillata, and associated Zooxanthellae*	Heat-shocked corals produced VLPs of diverse morphologies. Exposure of non-stressed zooxanthellae isolates to VLPs from heat-shocked zooxanthellae isolates resulted in cell lysis.	TEM, Flow cytometry	Davy et al. (2006)
Bleaching	*Porites compressa*	Abiotic stressors of bleaching (reduced pH, elevated nutrients, increased temperature) resulted in an increased abundance of herpes-like viral sequences.	Viral metagenomics and real-time PCR	Vega Thurber et al. (2008)
Bleaching	*Stylophora pistillata*	Heat shock of 31 °C in *Symbiodiniaceae*-enriched tissue increased the expression of a viral replication protein 114-fold.	Quantitative high throughput proteomics	Weston et al. (2012)
Bleaching	*Montastraea cavernosa*	Heat shock of 31 °C produced viromes with sequence similarity to dinoflagellate-infecting +ssRNA Heterocapsa circularisquama virus and dsDNA eukaryotic algae-infecting phycodnaviruses.	cDNA Viromes, Transcriptomics	Correa et al. (2013)
Bleaching	*Acropora sp.*	A viral outbreak associated with a local bleaching event was characterized by an abundance of viruses with morphologies similar to retroviruses, herpesviruses, and NCLDVs and a megavirus-like VLP in *Symbiodiniaceae*.	TEM, Viral metagenomics	Correa et al. (2016)
Bleaching	*Acropora tenuis*	Thermosensitive *Symbiodiniaceae* had decreased expression of +ssRNAV transcripts and increased expression levels of NCLDVs and anti-viral transcripts at higher temperatures. Thermal induction was restricted to the thermosensitive population.	Transcriptomics	Levin et al. (2017)
Bleaching	*Pocillopora sp.*	Viral metagenomes from bleached corals were enriched in eukaryotic virus sequences relative to non-bleached corals. Bacteriophage sequences were more abundant in metagenomes from non-bleached colonies than bleached colonies.	Electron microscopy (EM) and viral metagenomics	Messyasz et al. (2020)
Bleaching	*Pocillopora sp.*	*Symbiodiniaceae*-infecting +ssRNA viruses (dinoRNAVs) switched from a persistent to a productive infection mode with heat-stress. 17 dinoRNAV aminotypes were found only in heat-stressed corals and 22 were detected at higher relative abundances.	Ex situ aquarium experiment and major capsid protein (mcp) amplified cDNA sequencing	Grupstra et al. (2022)
Bleaching	*Porites lobata*	Over 90% (50/54) of corals had detectable dinoRNAV infections. Reef zone influenced the composition and richness of viral mcp amino acid types (‘aminotypes’). A reef-wide thermal stress event significantly increased aminotype dispersion, and this pattern was strongest in the colonies that experienced partial mortality.	Major capsid protein (mcp) amplified cDNA sequencing	Howe-Kerr et al. (2023)

(continued)

Table 5.1 (continued)

Disease/status	Host(s)	Evidence	Method	Reference
White Plague (WP)	*Favia favus*	A heat-sensitive "filterable factor" (<0.2 μm) from the tank water of a diseased coral in combination with a bacterial strain that alone is not pathogenic caused rapid lysis of F. favus.	Ex situ exposure experiments; bacterial culturing; size fractionation	Barash et al. (2005)
White Plague (WP)/ Bleaching	*Orbicella annularis (prev. Montastraea annularis)*	Eukaryotic circular Rep-encoding single-stranded DNA viruses and their associated satellites (SCSDVs) were abundant in WP-diseased tissues but not found in healthy tissues. NCLD sequences were most common in bleached corals.	TEM, metagenomics	Soffer et al. (2014)
White Plague (WP)	*Orbicella faveola*	Identified a number of upregulated genes in diseased corals that may play roles in the coral host's antiviral response or the viruses ability to replicate and evade the host.	Metatranscriptomics	Daniels et al. (2015)
White Plague (WP)/ Bleaching	*Pocillopora damicornis, Montipora aequituberculata, Porites compressa, and Acropora cytherea*	The genomes of *Vibrio coralliilyticus*, contain virulence gene encoding prophages and chromosomal pathogenicity islands that may drive its virulence.	Genomic analysis of bacterial genomes	Weynberg et al. (2015)
Porites White Patch (PWP)	*Porites sp.*	Icosahedral VLPs (<50 nm) were more abundant in coral and *Symbiodiniaceae* cells of PWP lesions than in healthy tissue.	TEM	Lawrence et al. (2014, 2015)
White Syndrome (WS)	*Acropora hyacinthus*	WS lesions were characterized by higher abundance, smaller size, and distinct morphology of VLPs relative to healthy coral tissues.	TEM, flow cytometry	Pollock et al. (2014)
Caribbean Yellow Band Disease/Yellow Blotch Disease (CYBD)	*Montastraea sp., Acropora sp., and Stephanocenia michillini*	VLPs (100–150 nm) resembling Phycodnaviruses were observed in the cytoplasm of isolated *Symbiodiniaceae* of diseased corals when they were subjected to temperature increases and bacterial pathogens.	TEM	Cervino et al. (2004)
Black Band Disease (BBD)/ White Plague Syndrome (WPS)	*Mussismilia braziliensis*	Caudovirales proteins were more abundant in BBD (0.9%, $n = 29$) compared to Healthy (0.4%, $n = 5$) and WPS (0.2%, $n = 2$).	Metaproteomics	Garcia et al. (2016)
Black Band Disease (BBD)	*Montipora hispida*	Relative abundances of three bacteriophage OTUs, affiliated to Cyanophage PRSM6 and Prochlorococcus phages P-SSM2, were significantly higher in BBD lesions than in healthy tissue. Viral beta diversities based on both operational taxonomic unit (OTU)-compositions and overall viral community compositions of assigned taxa did not differ statistically between the BBD-lesions and healthy coral tissue	Amplicon sequencing	Buerger et al. (2019)
Stony Coral Tissue Loss Disease (SCTLD)	*Colpophyllia natans, Dendrogyra cylindrus, Diploria labyrinthiformis, Meandrina meandrites, Montastraea cavernosa, Orbicella faveolata, Pseudodiploria strigosa, and Siderastrea siderea*	Elongated VLPs morphologically similar to filamentous +ssRNA viruses of plants termed anisometric VLPs (AVLPs) were associated with endosymbiont pathology in SCTLD-infected corals.	TEM	Work et al. (2021)

Overview of studies which attribute viral roles and/or associations to several different coral diseases sorted by the disease(s) of focus and the date of the study. Table includes the disease of focus, the coral host species included in the study, a summary of the evidence/findings, the method(s) used, and the reference from the tank water of WP-diseased corals which was necessary to cause disease in *Favia favus*, though to elicit rapid lysis of *F. favus*, it required the presence of an isolated bacterial strain that alone was not pathogenic. Thus, it may be speculated that this viral-sized filterable factor interacts with bacterial, rather than eukaryotic holobiont members (see bacteriophage section below). On the other hand, eukaryotic circular Rep-encoding single-stranded DNA viruses and their associated satellites (SCSDVs) are abundant in *Orbicella annularis* with white-plague disease, while they were completely absent from healthy specimens (Soffer et al. 2014). Metatranscriptomic analysis of diseased corals identified a number of upregulated genes that are speculated to play roles in the host's antiviral response, viral replication, and host evasion strategies (Daniels et al. 2015). A putative innate immunity factor that binds to ssRNA, dsRNA, or dsDNA viruses in antiviral responses (RNA helicase DDX60) was upregulated in the diseased tissue along with eukaryotic initiation factors and 40S/60S ribosomal activity which were speculated to play a role in the production of viral rather than host proteins. The upregulation of a gene involved in the prevention of viral replication or viral evasion of lysosomes (autophagy inhibitor GAPR-1) was also observed in diseased samples (Daniels et al. 2015).

A number of TEM studies have identified viruses associated with other coral diseases. Most viral-like particles (VLPs) found in white syndrome-infected tissues of *Acropora muricata* exhibited icosahedral morphology, lacked a tail and an envelope, and had a capsid diameter ranging from 120 to 150 nm (Patten et al. 2008). Larger VLPs (160–190 nm) were frequently observed, along with fewer VLPs smaller than 100 nm. Likewise, white syndrome lesions displayed a higher abundance of VLPs with smaller size and distinct morphology compared to healthy tissue in *Acropora hyacinthus* (Pollock et al. 2014). These findings suggest a possible link between the presence of these viral particles and the manifestation of white syndrome.

In *Porites* white patch disease, diseased tissue exhibited a higher abundance of icosahedral VLPs (<50 nm) compared to healthy tissue (Lawrence et al. 2014, 2015). VLPs resembling *Phycodnaviruses* increased in abundance in the cytoplasm of isolated *Symbiodiniaceae* from various coral hosts, including *Montastraea sp*., *Acropora sp.*, and *Stephanocenia michillini* suffering from yellow band disease/yellow blotch disease and exposed to high temperatures (Cervino et al. 2004). Stony Coral Tissue Loss Disease (SCTLD) emerged in 2014 in Florida and devasted Caribbean reefs in the following decade (Muller et al. 2020). Viral-like particles are associated with endosymbiont pathology in both SCTLD-affected and apparently healthy corals (Work et al. 2021). These particles, termed anisometric viral-like particles (AVLP), exhibited a morphology compatible with filamentous positive single-stranded RNA viruses of plants. The presence of AVLPs has led the proposal of a potential viral involvement in the pathogenesis of SCTLD in various coral hosts, including *Colpophyllia natans, Dendrogyra cylindrus*, *Diploria labyrinthiformis*, *Meandrina meandrites*, *Montastraea cavernosa*, *Orbicella faveolata*, *Pseudodiploria strigosa*, and *Siderastrea sidera* (Work et al. 2021). Yet, no causal association has been established in any of these examples.

5.2.4 Potential Benefits of Eukaryotic Viruses

While viruses are typically viewed through the lens of disease, they may also live in neutral or even beneficial association with eukaryotic hosts (Van Oppen et al. 2009). For example, compounding evidence suggests herpesviruses are present in most corals regardless of health status (Soffer et al. 2014; Pollock et al. 2014; Wood-Charlson et al. 2015). The viromes of *A. tenuis* and *G. aspera* also revealed viral genes encoding green fluorescent-like protein (GFP) chromoproteins, which may have antioxidant roles that counteract the effect of reactive oxygen species (Palmer et al. 2009; Bidle and Vardi 2011; Weynberg et al. 2017). Finally, the viromes of *P. verrucosa* and *G. aspera* contained sequences that matched Delta-thalatoxin genes from anemones (Oshiro et al. 2004). It was hypothesized that these toxin genes acquired by the coral-associated viruses benefit the coral host by assisting in food acquisition or defense, but these hypotheses have not been experimentally tested.

5.2.5 Viruses of Prokaryotes

The most abundant viruses observed in corals, double-stranded DNA viruses of the *Caudovirales* order, infect prokaryotes (Wood-Charlson et al. 2015; Weynberg et al. 2017; Cárdenas et al. 2020). These viruses can establish virulent and latent infections (Fig. 5.3) that modulate bacterial population densities, community structure, metabolism, and even pathogenicity (Brüssow et al. 2004; Vega Thurber et al. 2017; Breitbart et al. 2018; van Oppen and Blackall 2019). Mounting evidence also indicates that bacterial viruses participate in corals' ecological interactions with the rest of the reef, such as competitive interactions for benthic space (Roach et al. 2020) and biogeochemical cycles (Vega Thurber et al. 2017).

5.2.6 Modulation of Bacterial Community Structure

Animal mucosal surfaces are heavily colonized by microorganisms and play a vital role in protecting the host. In corals, viruses were 17-fold more abundant in mucus than in the surrounding seawater (Nguyen-Kim et al. 2015). The enrichment of viruses in coral mucus, similar to that demonstrated in mucosal surfaces of other organisms, suggests a symbiotic relationship between viruses and the animal hosts (Leruste et al. 2012; Nguyen-Kim et al. 2015). Phages may modulate the mucus bacterial community structure by selectively killing bacterial strains. Alternatively, structural modifications of bacterial communities may result in coral dysbiosis and diseases. In *Orbicella annularis*, phage consortia differed between bleached, diseased, and white plague-affected tissues (Soffer et al. 2014). Interestingly, the ratio of phage to eukaryotic viral sequences trended toward an increase in healthy tissues (19.21 ± 15.2) compared to WP-diseased tissues (4.77 ± 1.8). These results suggest that phages may play roles in the control of disease-associated bacteria.

In vertebrates, viruses interact with mucin glycoproteins in mucus in a mechanism named Bacteriophage Adherence to Mucus (BAM), resulting in the high lytic killing of bacteria (Barr et al. 2013). In the mucus, BAM is thought to act as a lytic barrier to pathogens, contributing to the coral immunity (Barr et al. 2013; Almeida et al. 2019). A second mechanism by which bacterial viruses may prevent invasion of the mucus microbiome is by the integration of phage DNA into the host genome (Fig. 5.2). These viral genomes bring in new functions to the bacterial cell, which can provide a competitive advantage to commensal bacteria (Silveira and Rohwer 2016; Wallace et al. 2024). The relative contribution of these two strategies for phage regulation of bacterial community composition is proposed to be spatially structured across mucosal surfaces due to differences in phage and bacterial densities and encounter rates in deep versus superficial mucus, where deep layers favor high lysis and crowded surface layers may favor phage integration through lysogeny (Silveira and Rohwer, 2016). Experimental work supports this hypothesis by demonstrating that prophages mediate competitive interactions between invading pathogens and resident bacterial strains, playing a major role in mediating bacterial colonization in corals (Wang et al. 2022). Here, the coral pathogen *Vibrio corallilyticus,* obtained from the gastric cavity of *Galaxea fascicularis,* releases hydrogen peroxide which triggers the lytic production of an integrated prophage of a non-toxigenic *Vibrio* sp. competitor, leading to the selective killing of the competitor (Fig. 5.1).

In a few cases where bacterial causative agents of coral diseases have been identified (Chimetto Tonon et al. 2017; Thompson et al., 2006), there have also been efforts to utilize phage therapy as a treatment (Efrony et al. 2007; Cohen et al. 2013; Atad et al. 2014) where lytic phages are used to target a pathogenic bacterium (Fig. 5.1). In *Favia favus* from Eilat in the Gulf of Aqaba, *Thalassomonas loyana* strain CBMAI 722T was isolated and identified to cause a WP-like disease (Thompson et al. 2006). In aquaria, the bacteriophage BA3 led to successful treatment if the phage was applied within one day of bacterial infection (Efrony et al. 2009). Treatment with phage also prevented the transmission of the disease from infected corals to healthy ones. Phage therapy of WP with phage BA3 remained successful when applied in situ and reduced the number of infected corals from 61% to 5% (Atad et al. 2014). *Vibrio coralliilyticus* has been associated with or found to cause white plague disease (Sussman et al. 2008, 2009; Chimetto Tonon et al. 2017) and bleaching (Ben-Haim et al. 2003). A lytic tailed phage, Bacteriophage YC, has been demonstrated to prevent photosystem inhibition of isolated *Symbiodiniaceae* that is typically induced by *V. coralliilyticus* strain P1 (LMG23696) (Cohen et al. 2013). This phage was also successful in preventing photoinactivation and tissue loss by *V. coralliilyticus* in juvenile corals, making it a potential candidate for phage therapy (Cohen et al. 2013).

5.2.7 Modulation of Bacterial Metabolism

Phages, the viruses of bacteria, can also provide competitive advantages to certain bacterial strains, pathogenic or commensal, by introducing auxiliary metabolic genes (AMGs) that modulate bacterial physiology (Breitbart et al. 2007). AMGs may function to improve the production of viral particles during the lytic cycle, but may also support biofilm formation, photosynthesis, carbon metabolism, and nucleic acid synthesis. In 101 corals sampled from the Central Red Sea, dsDNA phages transcribed genes involved in energy metabolism, bacterial motility, and photosynthesis, among other functions (Cárdenas et al. 2020). Homologs of photosystems genes are often found in phages (Sullivan et al. 2006; Ruiz-Perez et al. 2019) and have been identified in coral viromes worldwide (Marhaver et al. 2008; Weynberg et al. 2017). These photosystem gene homologs are hypothesized to alleviate or delay damage of *Symbiodiniaceae* caused by the impairment of photosystem II (PSII) and subsequent increase of reactive oxygen species (Van Oppen et al. 2009; Weynberg et al. 2017). Thus, phage contributions to holobiont functional diversity may scale up to coral health (Voolstra et al., 2024).

5.2.8 Lysogenic Conversion and Pathogenicity

Lysogenic conversion is a shift in bacterial phenotype triggered by the acquisition of genes brought in during prophage integration in the bacterial genome. Such conversion is a primary mechanism for the emergence of human and animal pathogens, as exemplified by *Vibrio cholerae*, which has its cholera toxin (CTX) encoded in a prophage (Wagner and Waldor 2002; Brüssow et al. 2004). Though this has not been directly demonstrated as a mechanism of coral disease, pro-

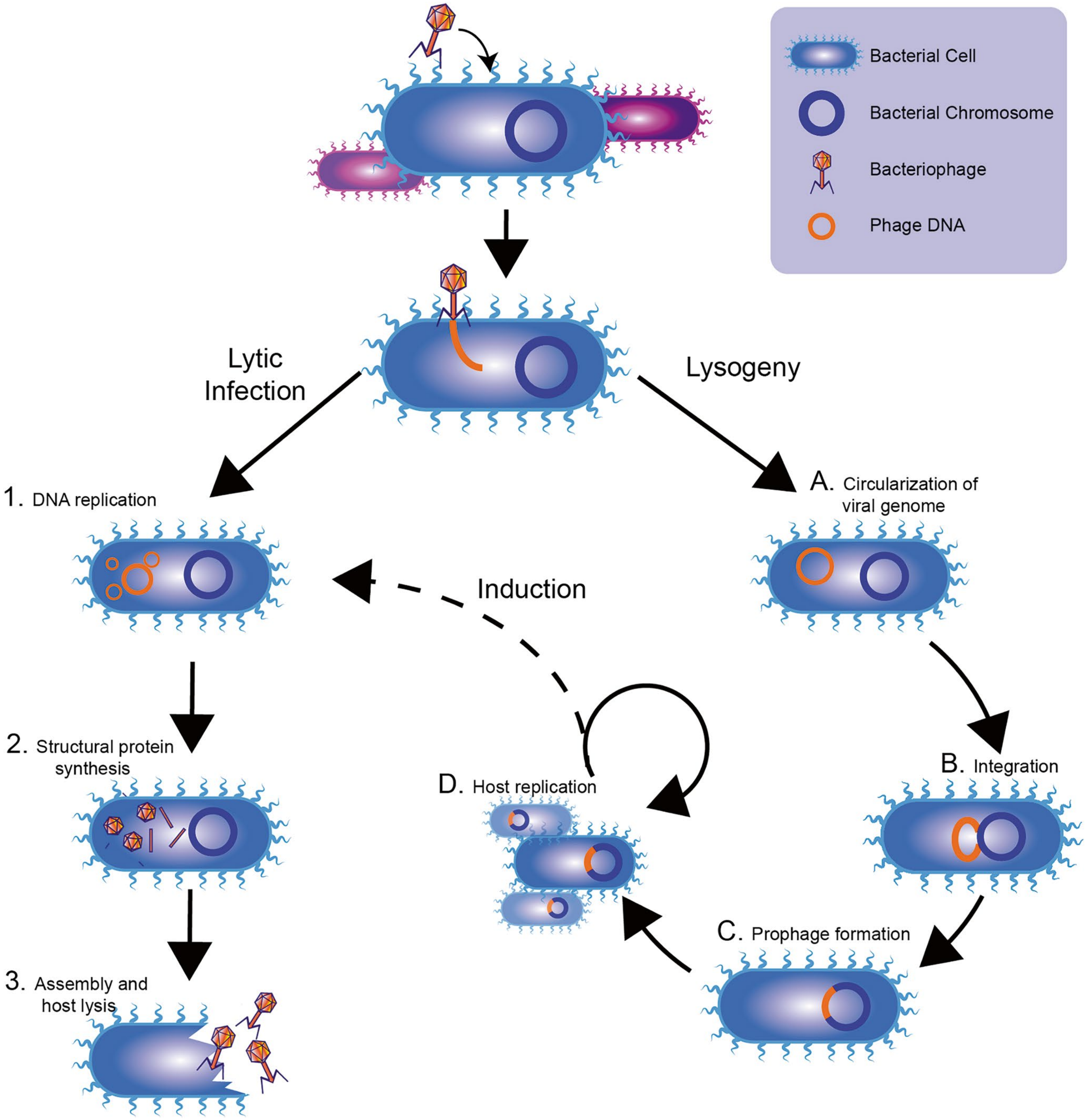

Fig. 5.2 Lytic and lysogenic infection. The most well understood infection strategies among prokaryotic viruses are lytic and lysogenic infections. (1–3) indicates the lytic cycle, and (A–D) indicates the lysogenic cycle. During lytic infection, (1) the virus makes several copies of its genome, and (2) using host machinery, structural proteins like the capsid and tail fibers are synthesized, and the viral DNA is packaged. (3) The virus uses holins and lysins to lyse the host cell and release progeny. During lysogeny, (A) the viral genome circularizes, (B) integrates into the host genome, and (C) forms a prophage. The prophage can replicate along with the host (D). It is possible for the prophage to induce and begin a lytic infection under various environmental stressors such as UV, heat shock, and DNA damage

phages encoding virulence genes are found in coral-associated bacteria. Phage genomes in *V. coralliilyticus* and *V. mediterranei* encode toxin genes resembling those found in pathogenic *V. cholerae*, as well as virulence factors located on chromosomal pathogenicity islands (Rubio-Portillo et al. 2014; Weynberg et al. 2015). These findings suggest that phages may contribute to the virulence of pathogenic vibrios through horizontal gene transfer. Consequently, targeting these elements becomes crucial in the screening of coral disease pathogens.

Black band disease (BBD), one of the earliest described coral diseases (Antonius 1973), is caused by a black microbial mat that creates an anoxic sulfide gradient lethal to coral tissue. Lysogenic infection by bacteriophages is thought to contribute to the virulence of this microbial consortium. High bacteriophage densities are found in cyanobacterial mats (Hennes and Suttle 1995; Voorhies et al. 2016). The genomes of BBD-associated cyanobacteria contain CRISPR-Cas defense systems against phages and putative virulence genes within prophage regions (Buerger et al. 2016). These findings are indicative of an evolutionary arms race between the bacteria and viruses where successful CRISPR defense of lytic phages by bacteria may promote BBD progression. The identified prophage-encoded virulence genes were related to lysozyme/metalloendopeptidases, lipopolysaccharide production, and a NAD-dependent epimerase/dehydratase. In *Montipora hispida* corals, Cyanophage PRSM6 and Prochlorococcus phages P-SSM2 show higher abundance in BBD-lesions, indicating their potential involvement in BBD pathogenicity (Buerger et al. 2019). Whether lytic or temperate, phages have the capacity to alter the functional roles of bacteria in BBD mats.

Compelling experimental evidence of the role of lysogenic conversion in coral diseases comes from white plague disease. A virus-sized "filterable factor" (< 0.2 μm) from the tank water of WP-diseased corals was necessary to cause disease in *Favia favus*, though to elicit rapid tissue loss, it required the presence of an isolated bacterial strain that alone was not pathogenic (Barash et al. 2005). The viral "filterable fraction" likely modulates the pathogenicity of BA-3 to elicit disease symptoms through lysogenic conversion. This hypothesis was later corroborated by the observed increase in the abundance of factors related to the regulation of phage gene expression and pathogenicity island genes in bacteria of WPD diseased corals (Daniels et al. 2015). Lysogenic conversion may represent a widespread mechanism for the emergence of pathogenicity in corals, explaining the remarkable difficulty in identifying bacterial pathogens using bacterial taxonomic markers that cannot detect prophages (Silveira et al. 2020).

5.3 The Reef Virome

Viruses are estimated to transfer 10^{24} genes per year in the global ocean (Rohwer and Vega Thurber 2009) and metabolically reprogram marine microbes, affecting how they interact with their ecosystem (Howard-Varona et al. 2020). There are approximately 10 million viruses per milliliter of seawater, most of which are bacterial viruses (Bergh et al. 1989). To put this number into perspective, the Great Barrier Reef is roughly 346,000 km^2 large (NASA Earth Observatory 2024), and by integrating this area with an average 1m benthic boundary layer and the average viral abundance in the reef seawater (10^6 virus per mL), we estimate that the GBR contains about 346,000,000,000,000,000,000,000 viruses. Because of the astronomical abundance of marine viruses, viral lysis can influence the global biogeochemical processes by regulating the population densities and physiology of microorganisms (Wilhelm and Suttle 1999). Viral lysis is estimated to account for 8 to 26 % of the daily heterotrophic bacterial mortality in oligotrophic marine surface waters (Lara et al. 2017; Fuhrman and Suttle 1993). Lysis by viruses releases bacterial biomass as organic matter into the environment, which can be used as fuel by heterotrophic bacteria and phytoplankton (Pomeroy 1974; Azam et al. 1983). In turn, these heterotrophs are a food source for small grazers and protists, allowing carbon to move up the food chain to higher trophic levels. This process of viruses adding to the dissolved organic matter pool is known as the viral shunt (Wilhelm and Suttle 1999). Another process, known as the viral shuttle, exports carbon to the seafloor in the form of marine snow (Guidi et al. 2016; Sullivan et al. 2017) (Fig. 5.3). In coral reefs, carbon and nutrients in the form of polymers and proteins form transparent polymeric particles that aggregate with mucus and fecal pellets and sink (Brocke et al. 2015; Torkelson et al. 2023; Huettel et al. 2006). The viral shuttle may contribute to this process by releasing particulate organic matter during lysis.

Viral abundances can vary significantly over short time scales and distances (Corinaldesi et al. 2003; Middelboe et al. 2006). For instance, while surface water typically contains 10^7 viruses per mL, in the deep oceans, the numbers can drop as low as 10^4 viruses per mL, and sediments can harbor approximately 10^9 viruses per g (Breitbart et al. 2008). Coral reefs have at least three distinguishable layers within the water column: the benthic boundary layer, the momentum boundary layer, and the diffusive boundary layer (Shashar et al. 1996; Barott and Rohwer 2012). These layers are created by the benthic modification of water flow and contain distinct chemical and physical characteristics, and therefore, different viral and bacterial densities (Crossland 1987; Wild et al. 2004; Barott and Rohwer 2012).

The benthic boundary layer, being the largest layer, operates on scales of meters, while the momentum boundary layer is typically measured within centimeters and the diffusive boundary layer within millimeters. In reefs with limited flow and complex reef structures, these layers may be much larger, facilitating the accumulation of dissolved organic carbon and dissolved organic matter, which in turn favors microbial proliferation. The microbial communities within the momentum boundary layers remain stable over short time scales of days (Silveira et al. 2017b) and have been observed to change in composition with proximity to the coral (Weber et al. 2019). This community, also referred to as the "coral ecosphere", exhibits an enrichment of genes asso-

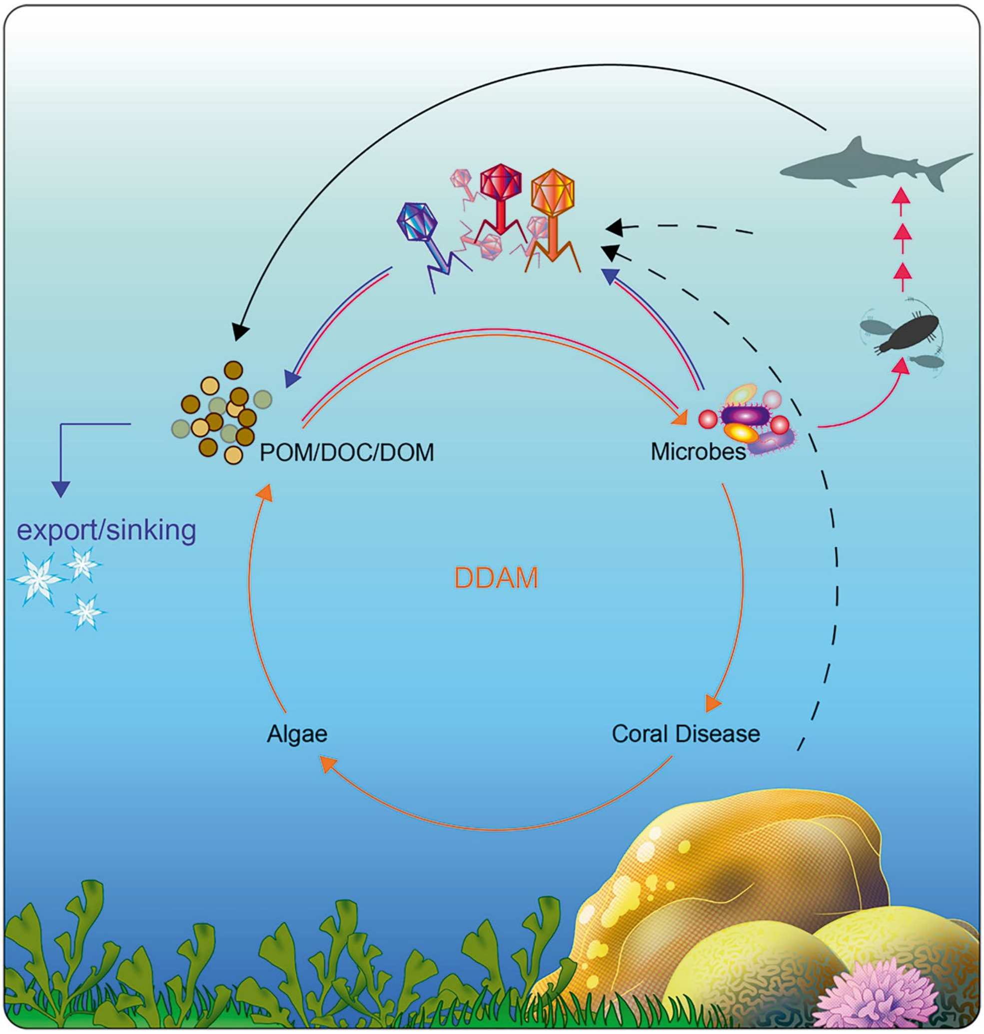

Fig. 5.3 Viral roles in reef health and biogeochemistry. The red arrow indicates the microbial loop transfer of biomass aided by viral lysis in lavender. The blue arrow on the left side indicates potential organic matter that sinks or is exported due to viral lysis. Orange arrows indicate the DDAM feedback loop or coral mortality

ciated with surface attachment and potential virulent lifestyles (Walsh et al. 2017; Weber et al. 2019). Notably, the abundance of viruses increases with proximity to coral colonies (Seymour et al. 2005; Vega Thurber and Correa 2011). Viral abundance was found to be highest within 4 cm of the coral surface (Seymour et al. 2005) and events of high lytic production have been observed in the momentum boundary layer (Silveira et al. 2017b). Viral lysis may influence nutrient regeneration and organic matter cycling rates, ultimately impacting the availability of food for coral reef benthic filter feeders (Silveira et al. 2017a).

Viral lysis also contributes to dissolved organic carbon (DOC) recycling in the reef, impacting the growth of copiotrophic bacteria. The DOC, Disease, Algae, and Microorganisms (DDAM) loop (Fig. 5.3) is a positive feedback loop where fleshy algae outcompete coral due to increased DOC availability and microbial growth that causes oxygen drawdown (Dinsdale et al. 2008; Barott and Rohwer 2012). DDAM fuels the global microbialization of coral reefs, as observed in a study of over 400 reef sites (Haas et al. 2016). The viral shunt has been hypothesized to increase DOC availability and exacerbate the DDAM loop, a process described as a viral 'revolving door' (Vega Thurber et al. 2017). However, recent findings from the Pacific suggest that the lytic top-down control of viruses on bacteria is actually associated with high coral cover, presumably by controlling detrimental bacterial overgrowth (Silveira et al. 2023). In this study, viral abundance was a better predictor of coral cover than bacterial abundance, herbivore, or large predator fish biomass. This pattern is reflected at small spatial scales in the interaction zones between corals and fleshy algae, where the virus-to-microbe ratio (a proxy for viral predation pressure over bacteria) is higher in areas of the coral colony far from the competition with algae (Roach et al. 2020). Viral transcripts are among the most differentially abundant in these interaction zones, suggesting the importance of viral activity in the coral-algae competition (Little et al. 2021).

Viral integration into the host genome also affects viral roles in coral reefs. Viral and bacterial abundances display a sublinear relationship, where the abundance of viruses does not scale proportionally with the abundance of bacteria (Knowles et al. 2016). High bacterial densities are associated with an increase in the abundance of temperate viruses, those that can integrate into the host genome through lysogeny rather than lysing the host and replicating. This pattern has been described as Piggyback-the-Winner, in contrast to the Kill-the-Winner dynamics where viral lysis is expected to increase with host availability (Knowles et al. 2016). High rates of viral integration at high microbial abundances may be caused by increased encounter rates, communication between phages and hosts, and benefits brought in through lateral gene transfer, which may also lead to the dominance of lysogenic bacteria through Make-the-Winner dynamics where viruses actually help host growth (Lara et al. 2017; Leonardo Moreno-Gallego et al. 2019; Shkoporov and Hill 2019; Jarett et al. 2020; Shkoporov et al. 2021; Sutcliffe et al. 2023; Luque and Silveira 2021). In addition to detrimental bacterial overgrowth, high rates of lateral gene transfer by phages through transduction incur a risk pathogenicity gene transfer and the rise of pathogens that may cause coral disease (Weynberg et al. 2017; Silveira et al. 2020; Messyasz et al. 2020).

5.4 Challenges and Future Directions

While we have gained significant understanding of the diversity of viruses inhabiting coral holobionts and coral reefs in the past 20 years, revealing the roles of these viruses remains a challenge, partially due to the technical limitations of studying viruses. The simple understanding of viral abundance dynamics across time and space is limited. A common method to quantify viral abundance is through epifluorescence microscopy. Due to their small size on the scale of around 100 nm, prokaryotic viruses cannot be seen with a regular compound microscope. Instead, a fluorescent DNA stain such as SYBR Gold is used to quantify viruses under an epifluorescence microscope (Noble and Fuhrman 1998). The main limitation of studying viruses using fluorescence microscopy is the lack of identity of the visualized viruses. Transmission Electron Microscopy, TEM is a method sensitive enough to reveal the structure of the capsids and tails of prokaryotic viruses and the membranes of eukaryotic viruses, but due to the methods of sample preparation, it cannot be used for quantitative analysis (Patten et al. 2008; Pollock et al. 2014). Both microscopy approaches are labor-intensive, and methods with high-throughput potential, such as flow cytometry quantification of viruses, are challenging and limited to seawater (Brussaard et al. 2001; Brussaard 2004). The development of a scalable and accurate method for field quantification of viruses in reef holobionts will represent a significant breakthrough in the study of reef viruses.

Metagenomics has revealed a staggering diversity of coral reef viruses (Vega Thurber et al. 2009). The lack of universal marker genes in viruses prohibits the use of amplicon sequencing surveys, such as 16S surveys for prokaryotes. To obtain viral metagenomes, viromes, the entire community needs to be sequenced and investigated bioinformatically. However, compared to prokaryotes, assembling viral metagenome-assembled genomes (MAGs) is a more difficult task. In part, this is due to their sequence diversity and high levels of recombination, which make it hard for assemblers to build scaffolds based on overlapping regions (Cárdenas et al. 2020). The differences in nucleic acids that viruses utilize is also a limiting factor, for instance, RNA viruses are frequently overlooked in metagenomic studies due to extraction protocols that focus on DNA. Additionally, the lack of viral hallmark genes and available viral reference genomes pose difficulties in estimating the completeness and contamination of viral genomes (Nayfach et al. 2020). Currently, viral genomes are generally identified within metagenomic data through either alignment-free algorithms that identify protein-encoding genes and match them with viral databases (Kieft et al. 2020; Guo et al. 2021; Chen et al. 2024). Viral databases like VOGDB, IMG-VR, and viral NCBI RefSeq contain cultured and uncultured viral genomes as well as viral protein sequences. Though these databases may have a bias towards dsDNA viruses (due to preferential sequencing of DNA), they can help identify major capsid proteins, tail fibers, or even hypothetical proteins found in viruses. Current viral identification software also use Hidden Markov Models (HMMs), neural networks and other deep learning approaches to identify and discriminate between viral sequence motifs (Kieft et al. 2020; Guo et al. 2021; Chen et al. 2024). Once a sequence is identified as viral, overlap and similarity features in the sequence such as tetranucleotide frequencies, codon usage, or GC content can be used to annotate genomes (Kieft et al. 2022). A Minimum Information about an Uncultivated Virus Genome (MIUViG) standard was developed to help standardize how we characterize viral genomes (Roux et al. 2018). This standard establishes that species-level viral genomes based on 95% average nucleotide identity and over 85% coverage, allowing the classification of taxonomic groups as well as what their functions are in each environment.

Perhaps the biggest challenge in the advancement of functional viromic studies in coral and other holobionts is obtaining enough viral nucleic acid (DNA or RNA) relative to host and other microbiome members (Wallace et al. 2024). Viral genomes are orders of magnitude shorter than that of bacteria and especially eukaryotes, so that in a sample that contains the complete holobiont community, few viral sequences can be obtained from regular metagenomic (or metatranscriptomic) sequencing (Wood-Charlson et al. 2015; Cárdenas et al. 2020). Metagenomic data generated from coral samples without pre-processing is mostly dominated by DNA from the coral host and *Symbiodiniaceae*, in addition to some other holobiont members, while the viral fraction can account for anywhere between just 0.16% and 0.40% of sequences (Wallace et al. 2024). A multitude of methods have been developed to circumvent these limitations, each with its own benefits and drawbacks (Table 5.2). For seawater samples, a common step to enrich viruses from complex communities is through filtration, using 0.45 µm or 0.22 µm filters to remove cellular organisms. Usually, a follow-up step is required to concentrate the viral fraction to ensure enough nucleic acid yield. For instance, Cesium Chloride gradients are a traditional method that yields viral particles with little cellular contamination (Breitbart et al. 2002). This method uses density gradients to select viruses by the physical properties of their capsids (Vega Thurber et al. 2009). However, this selects for specific viral groups. A less discriminate method to concentrate viruses is to use tangential flow filtration (TFF) or Polyethylene Glycol (PEG) to concentrate and precipitate viruses from solution (Colombet et al. 2007; Varona and Silveira 2023). While this method is relatively simple and cost-efficient for seawater samples, it may lose about 20% of the viruses (Göller et al. 2020), and is difficult to apply to coral tissues. An effective method for obtaining viruses from coral tissues uses sequential filtration and DNase treatments to remove *Symbiodiniaceae* and coral cells and DNA (Varona et al. 2023; Wallace et al. 2024). This method has been successful in increasing bacterial and viral representation in coral metagenomes by almost 10 times with low bias (Wallace et al. 2024).

In addition to identifying viruses and their genes, discerning which hosts these viruses infect is an even bigger challenge. One method of finding virus-host pairs is by searching for integrated viruses in host genomes. However, this only captures latent infections. Within Bacteria, CRISPR spacers are sequences that function in adaptive immunity and can be matched with free viruses to identify virus-host pairs. However, CRISPRs are difficult to assemble due to their repeats and are only present in about 10% of cultivation-independent microbial genomes (Burstein et al. 2016). Additionally, neither CRISPR nor prophage matches will identify whether these infections are actively occurring or were acquired in the past. Other methods, such as matching tRNAs (within prokaryotes) or inferring from sequence identity with genomes in available databases can be used to predict the virus's host, however, these methods have lower taxonomic resolution. Meta-HiC is a proximity-ligation method that cross-links DNA within a cell (Bickhart et al. 2019; Uritskiy et al. 2021; Marbouty et al. 2021; Hwang et al. 2023). A chemical agent, like formaldehyde, is used to crosslink DNA in physical proximity, such as the viral DNA and the host DNA during infection. This viral-host DNA hybrid can then be sequenced together as a chimeric sequence, and through bioinformatic analyses, viruses can be matched with their host. This method has revealed active infections of heterotrophic bacteria in coral reef boundary layers in the Caribbean, where Gammaproteobacteria made up 19.4% of the total community but 32.9% of the infected community (Varona et al. 2024). Interestingly, this method revealed that most active viruses were not the most abundant members of the viral community but rather those viruses ranging in mid to low abundances. Furthermore, their preferential target towards heterotrophic bacteria which can act as opportunists during coral disease, may explain why high viral frequency is related to healthy coral reefs (Silveira et al. 2023). A limitation of proximity ligation is that it may not be

Table 5.2 Methods applied to the study of viral diversity in corals

Method	Function	Benefits	Limitations
Cesium Chloride (CsCl) Density Gradients	Fractionation by density gradients	Highly purified viral fraction	Excludes nucleic acids from large viruses and hosts, bias toward certain viral groups, may require amplification methods which incur further biases
Polyethylene Glycol (PEG)	Highly soluble chemical that crosslinks with protein structures	Time and cost efficient	~20% loss of viral fraction
Tangential Flow Filtration (TFF)	Concentration of large volumes	High amount of nucleic acid yield	Better suited for large amounts of liquid samples
Amicon Concentration	Concentration of small-to medium volumes	High nucleic acid yield, low bias	Requires repetition for sufficient nucleic acid yield
Host DNA Depletion	Disrupt the larger coral host and algal symbiont cells and degrade their DNA prior to extraction and sequencing	Enriches for both bacteria and viruses in a single metagenome	Labour-intensive, variable results with different coral species
Whole Sample	No pre-processing	No selection bias	High proportion of coral and *Symbiodiniaceae* sequences

Table encompasses the functions/targets, benefits, and limitations of methods used to describe viral diversity in corals able to ensure that viral replication and lysis was successful for each linked host, and is not informative for eukaryotic viruses. Another culture-dependent approach, single-cell sequencing, is useful for ukaryotic hosts, but much lower throughput (Džunková et al. 2019).

Among viruses that infect culturable bacteria, sequence-independent approaches, such as plaque assays or viral tagging, can also be used to track viral infection. Plaque assays have been the most robust method to infer virus-host pairs. For this, the prokaryotic host is grown on as a lawn on a plate, a virus of interest is added, and if it infects, it will lyse the host and create a clear "plaque". However, the challenge in this relies on being able to isolate the virus and the host. With less than 1% of prokaryotes being currently culturable, this remains a large challenge. Yet, the benefits are also enormous: with a virus and its host in culture, experimental studies can mechanistically reveal viral functions on coral health (Wang et al. 2022). A 96-well plate has been developed to expedite the cultivation of reef holobiont viruses for known, culturable hosts without the need for plaque assays on a solid medium. This approach takes advantage of crashes in liquid cultures caused by lytic infection and detected by a plate reader (Veglia et al. 2021). Viral-Tagging offers more flexibility by using DNA-binding fluorescent dye to tag viruses which then can infect hosts. The stained viruses can then be detected and sorted using flow cytometry to capture virus-tagged cells (Jang et al. 2022).

There are a number of other methods, such as using digital PCR to amplify known regions of the virus and detect it in other cells (Tadmor et al. 2011), or single amplified genomes coupled with cell sorting (Labonté et al. 2015). Despite the growth of methods to study viral ecology, most of these techniques have not yet been tested on corals or reef inhabitants. More than ever, the technological advances in the last decades have opened doors to exploring viruses' roles in corals and their reefs. These technologies will allow us to investigate questions about the diversity and distribution of viruses, who these viruses are infecting, what genes they are transferring, and give a better understanding of their role in coral reefs.

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Fungi and Fungi-Like Entities

6

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Abstract

Coral microbiology research has long focused on the composition and functional roles of prokaryotic organisms, but microeukaryotic communities, including the enigmatic fungi remain a poorly understood “black box” within coral and other holobionts. Here, we summarise what is known and hypothesised about the diversity, functional traits and potential, and chemodiversity of coral- and reef-associated fungi and fungi-like organisms (FLOs). Finally, we briefly outline the challenges associated with the characterization of marine fungi and provide a perspective for future studies to elucidate the biology, chemical ecology, and organismal interactions of marine fungi and FLOs within coral reef holobionts and their potentially far-reaching roles in coral reef ecosystem functioning and health.

Keywords

Coral reefs · Microeukaryotes · Fungi · Fungi-like organisms · Inter-kingdom interactions · Nutrient cycling · Microbial ecology · Cross-talk

6.1 Introduction

Tropical coral reefs are hotspots of marine productivity and diversity, and as such often referred to as the ‘rainforests of the sea’. Their main ecosystem engineers, the reef-building corals, are complex holobionts consisting of the cnidarian animal host and a plethora of prokaryotes and microeukaryotes (Rohwer et al. 2002; Roik et al. 2022; Pogoreutz et al. 2020). The remarkable ecological success of coral reefs over hundreds of millions of years (Frankowiak et al. 2016) is attributed to the mixotrophic coral-algal symbiosis, an efficient reciprocal nutrient-exchange relationship, which constitutes adaptation of corals to the oligotrophic (nutrient-poor) tropical waters they typically inhabit (Falkowski et al. 1993; Muscatine and Porter 1977). Our understanding of the coral-algae symbiosis builds upon decades worth of functional research (Davy et al. 2012; Pogoreutz et al. 2020). In contrast, the roles and interactions of other coral- and reef-associated microbes remain poorly understood. However, increasing numbers of studies suggest a functional importance of certain functional groups of prokaryotes and microeukaryotes in the health and resilience of coral reef holobionts (Doering et al. 2021; Cárdenas et al. 2022; Pogoreutz et al. 2022; Tandon et al. 2020; Ngugi et al. 2020; Santoro et al. 2021; Ziegler et al. 2017; Pogoreutz and Ziegler 2024) (among many others). In this light, substantial efforts all around the globe are currently channelled into the development and ground-truthing of probiotic applications and other microbial-based intervention strategies for coral reef bioremediation and reef restoration purposes (Rosado et al. 2018; Doering et al. 2021; Dungan et al. 2021; Peixoto et al. 2017; Maire and van Oppen 2021; Delgadillo-Ordoñez et al. 2024; Damjanovic et al. 2019; Buerger et al. 2020; Zhang et al. 2021b).

Besides bacteria, the under-investigated microbes of corals and coral reefs include diverse viruses, archaea, and microeukaryotes, among them enigmatic fungi and fungi-like organisms (FLOs) (Thurber et al. 2009; Roik et al. 2022;

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Cárdenas et al. 2020, 2022; Bonacolta et al. 2023; Clerissi et al. 2018; Campo et al. 2020). Beyond fungi and FLOs, we can broadly distinguish reef-associated microeukaryotes into photo- and heterotrophic groups (Bonacolta et al. 2023). Apart from the well-known Symbiodiniaceae (LaJeunesse et al. 2018), reef-associated (micro)eukaryotic phototrophs include diverse algae, such as Chlorophyta (green algae) and Rhodophyta (red algae), the latter including the crustose coralline algae, some of which have been identified as inducers of larval settlement and metamorphosis in different marine invertebrates, including corals (Heyward and Negri 1999). Heterotrophic reef-associated microeukaryotes include Fungi and FLOs (different groups of Stramenopiles, specifically the Labyrinthulomycetes and Oomycetes), alveolates commonly referred to as Apicomplexa, including the two chromerids *Chromera velia* and *Vitella brassicaformis* (Moore et al. 2008; Oborník et al. 2012; Mohamed et al. 2018), and ciliates (Sweet and Séré 2016, reviewed in Bonacolta et al. 2023; Ainsworth et al. 2017). Of these understudied microeukaryotes on the reef, fungi and FLOs together have received attention for their substantial chemodiversity which makes them prime targets for natural products chemistry, but also for their potentially opportunistic and pathogenic lifestyles (Roik et al. 2022). Considering the importance of terrestrial fungi as ecological driving forces that shape nutrient cycles and thereby entire ecosystems (Coleine et al. 2022), knowledge of the diversity, functions, and inter-kingdom interactions of coral- and reef-associated fungi may be important for our understanding of coral holobiont- to coral reef ecosystem-level responses to global environmental change (Roik et al. 2022; Cavicchioli et al. 2019).

6.2 Occurrence, Prevalence, and Putative Roles of Coral Reef-Associated Fungi

Cell numbers and biomass of marine fungi are typically low in oligotrophic environments such as the open ocean, and most likely fungal cells are found in association with particles (Wurzbacher et al. 2010). In this light, it has been previously proposed that oligotrophic coral reef waters may support only low to moderate biomasses of marine fungi, and reef-associated fungi may be mostly associated with benthic reef substrata and organisms (Roik et al. 2022). Fungal niches on the reef may include stagnant microenvironments characterised by steep gradients of light, oxygen, pH, and nutrients (Risk and Muller 1983; Schlichter et al. 1997; Wangpraseurt et al. 2012) (summarised in Roik et al. 2022; Pernice et al. 2019). As such, Roik et al. (2022) proposed that environmental and inter-kingdom interactions of fungi on the reef may be primarily relevant in very specific habitats, such as in benthic reef substrata (the reef framework and rubble), reef sediments, and in the interstitial spaces of (in)vertebrates, but also on skin and surfaces and in tissues, the gut, skeleton and shells of benthic reef organisms, and, lastly in association with pelagic organisms, in particular microalgae (Golubic et al. 2005; Wong Chin et al. 2021; Pernice et al. 2019; Cárdenas et al. 2022; Gao et al. 2008; Roik et al. 2022; Bentis et al. 2000; Liao et al. 2023). Benthic habitat-forming marine invertebrates, Cnidaria (scleractinian and octocorals) and sponges in particular, have been in the focus of microbiome research for more than two decades, although most studies focused on their associated bacterial communities. A recent study has found that cryptic and zoobenthos, including microscopic Annelida, Platyhelminthes, Chaetognatha, Kinorhyncha, Nematoda, and small crustaceans (Holt et al. 2022; Roik et al. 2022) host a surprising diversity of protists and fungi. However, care must be taken when interpreting sequencing data of marine fungi. While marine fungi may be implicated in inter-kingdom interactions within animal, plant, or macroalgae holobionts (Roik et al. 2022), we cannot currently rule out that a proportion of fungi found associated with aquatic and marine filter- and detritus-feeding invertebrates may simply have been ingested. Finally, some fungi detected in marine samples may not even be of aquatic origin, but rather contamination from terrestrial run-off or dust deposition events. Hence, the study of fungal occurrence, community diversity, and prevalence on coral reefs, along with the potentially diverse functions of fungi associated with marine invertebrates remains a challenge and will require multi-faceted approaches (Holt et al. 2022; Roik et al. 2022).

The scarcity of metabarcoding datasets for coral- and reef-associated fungi partially reflects the well-known constraints of phylogenetic markers and/or genomic databases available, in particular for fungi in the marine realm (Frau et al. 2019; Rabbani et al. 2021). In addition, there is the prevailing issue of high proportions of host nucleic acids disproportionately skewing sequencing coverage and cross-amplification with host DNA in host-associated microbiomes in '-omics' datasets (Pogoreutz et al. 2022; Feehery et al. 2013; Pereira-Marques et al. 2019). Furthermore, across the few studies available, there is little consistency with regard to the coral host compartments that were sampled (skeleton, tissues, mucus; sometimes pooled, sometimes separated), and the biomarkers used. Here, studies employed different hypervariable regions on the small and large ribosomal subunits (SLU and LLU, respectively) and the internal transcribed spacers ITS1 and ITS2 (Rabbani et al. 2021; Chavanich et al. 2022; Góes-Neto et al. 2020; Lifshitz et al. 2020; Paulino et al. 2020; Bonthond et al. 2018; Amend et al. 2012; Wegley et al. 2007) (summarised in Roik et al. 2022); refer to Sect. 6.7 'metabarcoding and metagenomic approaches' below for further detail).

In this context, although plausible, we can not currently draw general conclusions on fungal communities in reef-building corals across different holobiont compartments. Several studies indicate that fungi may be ubiquitous and wide-spread members of reef-building coral holobionts, as affiliated sequences were recovered from corals across the entire distribution range of coral reefs (Roik et al. 2022). Fungal communities associated with coral holobionts respond to environmental stress (Amend et al. 2012), but reports on fungal community responses in corals during heat stress and bleaching are scarce and inconclusive. While some studies have reported seemingly 'stable' fungal community compositions even during coral bleaching (Longley et al. 2023), others report increased fungal diversity and relative abundance of putative pathogenic fungi in bleaching-susceptible corals (Chen et al. 2024). Such seemingly contradictory observations highlight the likely complex and multifaceted association between corals and their fungal communities. Across these studies, coral-associated fungal communities were commonly dominated by unicellular and filamentous fungi belonging to the phyla Ascomycota, Basidiomycota, and Chytridiomycota, with other Phyla making up smaller proportions (Figs. 6.1 and 6.2) (Thurber et al. 2009; Amend et al. 2012; Lifshitz et al. 2020; Paulino et al. 2020; Cárdenas et al. 2022; among others). Similarly, associations of octocorals and fungi have been described. The culturable fungal diversity of octocorals includes the genera *Aspergillus* and *Penicillium* which have been commonly isolated from different species of octocorals across ocean basins, specifically in the Caribbean (Toledo-Hernández et al. 2008; Zuluaga-Montero et al. 2010), in the Eastern Pacific (Soler-Hurtado et al. 2016), and in tropical Asia (Koh et al. 2002; Zhang et al. 2012). Further, members of the genera *Cladosporium*, *Tritirachium*, *Nigrospora* and *Fusarium* have been isolated from different species of octocorals (Koh et al. 2002; Toledo-Hernández et al. 2008; Zhang et al. 2012; Zuluaga-Montero et al. 2010; Soler-Hurtado et al. 2016). Similar to reef-building corals, octocoral-associated fungal community compositions are affected by the local environment (Zhang et al. 2012; Zuluaga-Montero et al. 2010), although further research will be required for a more complete understanding of drivers of community dynamics.

Overall, the association of fungi with Cnidaria might be less common than with other marine invertebrates hosts. A recent study found that fungal sequences were recovered from less than half of the cnidarian samples investigated, whereas fungal and FLO sequences were obtained from the large majority of samples from other filter-feeding marine invertebrates (Holt et al. 2022). Hence, cnidarian associations with fungi may be random or opportunistic, and not necessarily be of functional relevance. Alternatively, the results of this study could have been driven in part by the technical biases and challenges of obtaining DNA from different types of organisms, tissues, and cell types (e.g., host and associated fungi). Interestingly, the same study identified a *Cladosporium* species (commonly but not exclusively associated with phytoplankton in the open ocean (Letcher and Powell 2018) that was common across all investigated marine invertebrates except Cnidaria (Letcher and Powell 2018). As such, it might be possible that the presence of some of the fungal sequences in samples of filter-feeding invertebrates could be the signature of feeding activity and ingestion of fungi along with other food items from the water column rather than evidence for a stable invertebrate-fungi association. In this regard, the absence of (predominantly pelagic) fungal sequences in cnidarian samples might simply point to a more selective feeding behaviour compared to the other filter feeders included in the study (Letcher and Powell 2018). In a nutshell, the diversity of possible interpretations highlight that well-designed studies on the occurrence and prevalence of fungi on marine holobionts are urgently needed.

On this note, it cannot be ruled out that fungi and their spores ingested by marine filter-feeding invertebrates may not always be digested, and as such may end up persisting in the guts of different holobionts, where they might even establish themselves exploiting available food sources. Thereby, these fungi could contribute to decomposition and digestion processes of ingested food items. Among 'gut' microbiomes of coral reef organisms, the intestinal microbiomes of fish have received the most attention over the past years. Here, quite a number of studies have examined the bacterial compositions of fish guts (Smriga et al. 2010), whereas only one investigated the intestinal mycobiome of coral reef fish identifying a culturable community predominantly dominated by members of Ascomycota and Basidiomycota (Liao et al. 2023). Half of these isolated fungi exhibited anti-microbial activity against known pathogenic microorganisms in vitro. The most remarkable anti-microbial activity was reported from the isolates classified as similar to *Schizophyllum commune* and *Aureobasidium pullulans*, the latter of which also exhibited anti-mycotic effects against *Aspergillus* species in vitro (Liao et al. 2023).

Finally, marine sponges and their microbes have been of interest for natural products chemistry for a long time. Associated fungi have been of particular interest due to their enormous bioactive potential and chemodiversity. Sponges are typically dominated by anamorphic Ascomycota, but also harbour a diversity of Basidiomycota. Among these, yeasts appear to be fairly common. Overall, sponges associate with fungal species of diverse lifestyles, including pathogens and potential endosymbionts of to-date-unknown functions. Some of these fungi appear to be maternally transmitted, and hence presumed to be mutualistic symbionts of sponges (Maldonado et al. 2005). Most obviously, as we know from numerous bioprospecting efforts, sponges harbour an appreciable diversity of novel (Höller et al. 2000), obligate marine fungi. These fungi are exclusively recovered

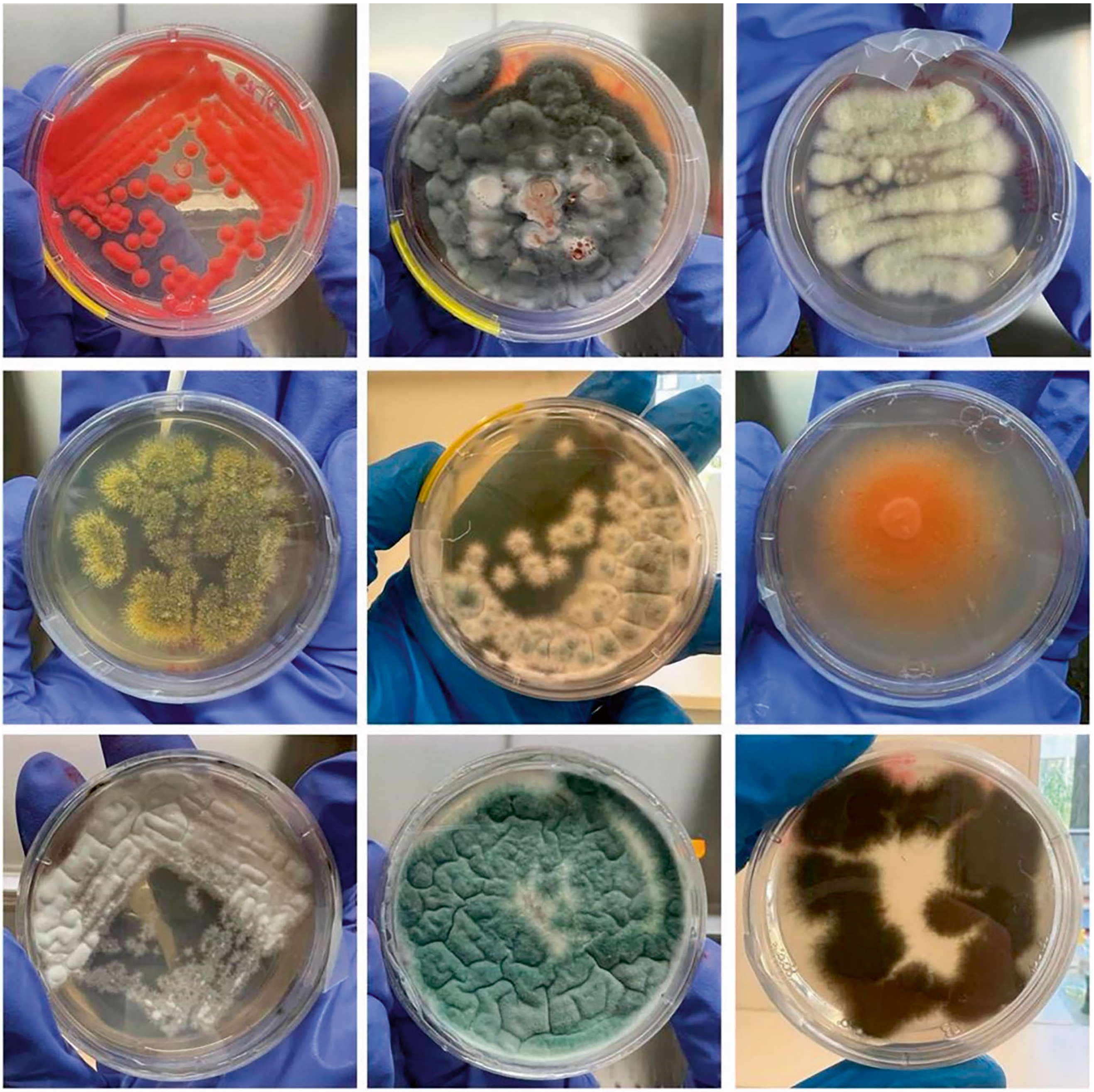

Fig. 6.1 The macroscopic diversity of marine-derived fungal isolates from the temperate cnidarian photosymbiotic sea anemone *Anemonia viridis* include both yeast-like and filamentous phenotypes (images: Claudia Pogoreutz)

from marine environments and/or are genetically only distantly related to fungal sequences obtained from terrestrial sources, suggesting true 'marine phylotypes' (Gao et al. 2008). On the other hand, the lipophilic yeast *Malassezia*, members of which are found in diverse habitats ranging from the skin of humans and domestic animals to the deep sea, marine mammals and corals, appears to be a fairly common associate of different sponges, and has been suggested to be potentially relevant across different marine holobionts (summarised in Raghukumar 2017). Similar to coral-associated fungi, the ecological roles and functions of sponge-associated fungi remain to be further elucidated.

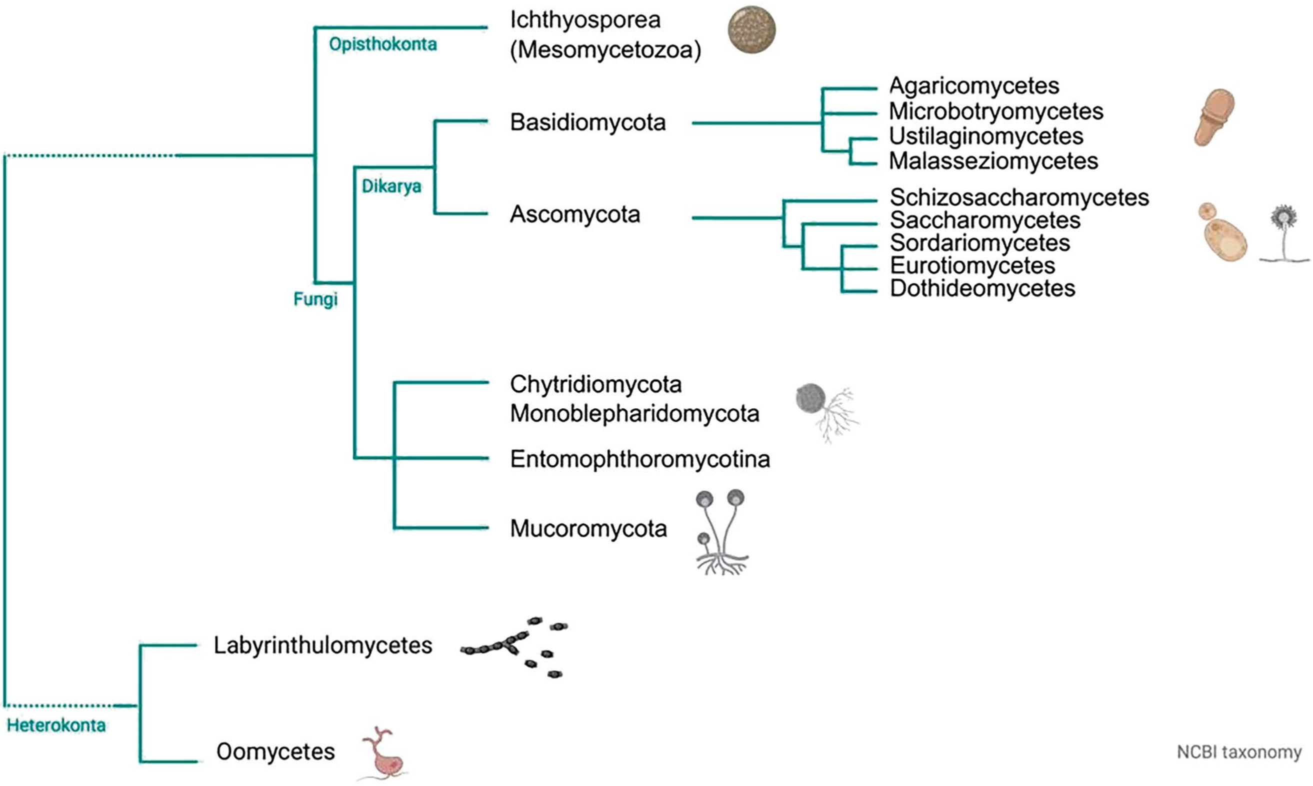

Fig. 6.2 Pruned phylogenetic tree displaying consistently reported fungal phyla (and classes for Ascomycetes and Basidiomycetes) and Fungi-like organism taxa across coral studies (based on NCBI taxonomy; tree generated using *phyloT v2* (Letunic 2024); figure modified in *BioRender*). Adapted from Roik et al. (2022)

6.3 Fungi-Like Organisms (FLOs) and Other Microeukaryotes on the Reef

FLOs are affiliated to the group of marine stramenopiles, or Heterokonta and include the zoosporic Peronosporomycota (formerly Oomycota) and Labyrinthulomycetes (Fig. 6.2). Sequences affiliated to these organisms are commonly recovered from coral and other marine samples in metabarcoding and metagenomic datasets (Cárdenas et al. 2022; Bonthond et al. 2018; Ettinger and Eisen 2020), and some members of this group are amenable to cultivation (Raghukumar and Raghukumar 1991; Ben-Dov et al. 2009; Siboni et al. 2010; Burge et al. 2012; Harel et al. 2008; Raghukumar 1991). Their specific functions in marine organisms remain poorly understood, but they are commonly encountered as 'contaminants' that proliferate and persist in primary cell cultures of diverse marine invertebrates, including cnidarians, sponges, and tunicates (Frank et al. 1994; Blisko 1998; Rinkevich 1999; Rabinowitz et al. 2006), highlighting their ubiquity in marine animal holobionts.

Among this elusive but extensive diversity of FLOs, Labyrinthulomycetes have probably been the most widely investigated. Labyrinthulomycetes have the ability to synthesise a diversity of bioactive compounds including polyunsaturated fatty acids, docosahexaenoic acid, squalenes, and carotenoids, and have been explored for their industrial biotechnological potential as a new source material for biofuels and lipid biofactories (summarised in Qarri et al. 2021). Beyond this, little is known about the functions of marine Labyrinthulomycetes other than them being saprobionts, i.e. degraders of organic material (Tsui et al. 2009; Richter n.d.). Another poorly studied group of FLOs on the reef are the Oomycota. The availability of a handful of cultures obtained from the seagrass *Zostera marina* affiliated to the Genus *Halophytophthora* suggests that at least some Oomycota associated with marine benthos are culturable (Ettinger and Eisen 2020), and as such could be used for genomic and functional exploration.

6.4 Fungal Traits of Potential Relevance on Coral Reefs

The ocean with its distinct physicochemical environments differs very much from terrestrial ecosystems in terms of biotic and abiotic factors. This suggests a substantial adaptive capacity and versatility of fungi to rapidly colonise new habitats and hosts (summarised in Roik et al. 2022). For a

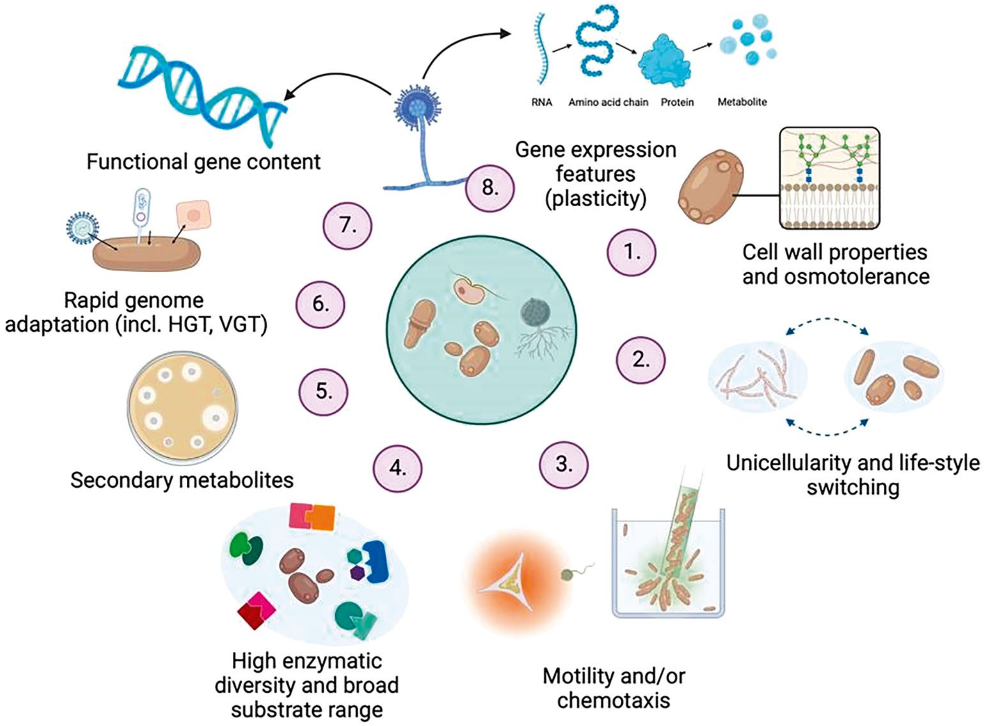

Fig. 6.3 Functional traits of marine fungi proposed to be relevant for dispersal, habitat colonisation, and adaptation to marine hosts and environments, including corals and coral reefs. Figure created in BioRender

better understanding of how fungi may have adapted to aquatic ecosystems in general, and to marine and reef ecosystems in particular, it is important to consider their main functional traits (Fig. 6.3). Importantly, while these traits have so far been explored mainly in terrestrial pathogenic fungi lineages, they may be equally relevant in the dispersal, radiation and adaptation of marine and coral reef-associated fungi (Roik et al. 2022).

6.4.1 Cell Wall Properties and Osmotolerance

Salinity is considered a main barrier to fungal dispersal, activity and growth (El Baidouri et al. 2021). As such, adaptation of fungal cell walls to ocean life include efficient osmoregulation and maintenance of cellular homeostasis. Fungal cell walls consist of multiple layers of polysaccharides which render the cell water absorbent and stable (Szaniszlo and Mitchell 1971; Durán and Nombela 2004). Interestingly, cell wall compositions differ only qualitatively in terms of carbohydrate, amino sugar, amino acid and fatty acid composition between terrestrial and marine lineages (Szaniszlo and Mitchell 1971; Ravishankar et al. 2006; Plemenitaš et al. 2014; Danilova et al. 2020). Marine fungal cell walls exhibit specific fatty acid modifications that permit high cell wall integrity and fluidity (Turk et al. 2004; Kogej et al. 2007; Gostincar et al. 2009). Finally, and akin to terrestrial lichens living in high UV environments, marine fungal cell walls contain the pigment melanin, which increases resistance to various environmental stressors by increasing strength and rigidity of cell walls, osmotolerance, hydrostatic resistance, and UV radiation (Casadevall et al. 2017). Osmotolerance varies in different marine fungi species. For instance, a particularly high salt tolerance is known from the model organism *Hortaea werneckii*, which can cope with and even grow in environments up to 5 M NaCl (Plemenitaš et al. 2014). This organism accumulates ions and efficiently uses the high-osmolarity-glycerol (HOG) signal transduction pathway in response to hyperosmotic stress (Turk and Plemenitas 2002; Kogej et al. 2007). In addition to glycerol, various halophilic and halotolerant fungi deploy diverse osmolite pools made up of saccharides, polyols, melanin, mycosporine-like amino acids, and UV-absorbing compounds (Kogej et al. 2006; Ravishankar et al. 2006; Danilova et al. 2020). These osmolyte pools are fungi species-specific and vary with growth phase (Kogej et al. 2007) and in response to the abiotic environment (Gonsalves and Nazareth 2020).

6.4.2 Dimorphic Switching to Accommodate Lifestyle Changes

Plasticity of morphological and lifestyle traits permits fungi to conquer new environmental niches and host organisms through different strategies (Větrovský et al. 2019). Dimorphic switching, i.e. the reversible switch between multicellular hyphae (filamentous) and a unicellular 'yeast' lifestyle renders some fungi particularly successful (Boyce and Andrianopoulos 2015). Dimorphic switching is primarily observed in terrestrial ascomycetes, and triggered by environmental cues such as temperature (Pasricha et al. 2017; Francisco et al. 2019). Many dimorphic fungi are infectious in only one of the two stages. The switch can be accompanied by metabolic reprogramming and cell wall compositional remodelling to evade detection by the host immune system, enabling invasion and infection of host tissues and subsequent pathogenesis (Trofa et al. 2008; Nadal et al. 2008; Klis et al. 2009; Nagy et al. 2017). While such behaviour or the general infection biology of marine fungi in corals remain unknown, it has been documented that fungi attempt penetration of coral tissues from the calcareous skeleton underneath (Bentis et al. 2000), and that fungal hyphae are abundant in the tissues of stressed, morbid, and diseased corals (Strake et al. 1988; Work et al. 2008). While dimorphic switching has not yet been described in coral reef environments, such behaviour could constitute one potential strategy to colonise diverse environments and hosts (Roik et al. 2022).

6.4.3 Motility, Chemotaxis, Attachment

Some aquatic fungi, including the ancestral zoosporic lineage of Chytridiomycota, exhibit a degree of motility. These organisms swim actively, permitting them to home in on new substrates and hosts (van Hannen et al. 1999). Such behaviour is modulated by chemotaxis towards chemical cues, typically amino acids and carbohydrates (Muehlstein et al. 1988; Scholz et al. 2017). Most aquatic fungi however are non-motile. They typically attach to and grow on substrates. Attachment strategies are diverse and include the production of mucilaginous sheaths, surface proteins called flocculins (Ogawa et al. 2019), and extracellular polysaccharides (Kimura et al. 1998). In aquatic yeasts, for instance, a group of carbohydrate binding cell wall proteins called lectins are primarily implicated in the aggregation and substrate adhesion. These lectins bind to polysaccharides on the cell walls of hosts or to detritus (Singh et al. 2011).

6.4.4 Diverse Nutrient Acquisition Strategies Through Enzymatic Diversity

Fungi are best known for their diverse and efficient nutrient acquisition strategies. These are rooted in a diverse battery of exoenzymes, likely resulting in high substrate affinity and broad substrate range (Newell 1996; Zhao et al. 2014b; Hagestad et al. 2021; Thomas et al. 2022). These exoenzymes include diverse carbohydrate-active enzymes, or CAzymes (Lange et al. 2019) which can degrade lignins and other algal polysaccharides, (hemi)celluloses, tunicin (Kohlmeyer and Kohlmeyer 2013), and chitin (Tang et al. 2006), the most abundant polysaccharide in the ocean (Souza et al. 2011). Many marine substrates are either unique (i.e., not present on land or in freshwater), or have additional sulphate groups which need removing prior to substrate breakdown (Janusz et al. 2017; Schultz-Johansen et al. 2018; Barbosa et al. 2019; Kappelmann et al. 2019). Unique substrates include algal polysaccharides such as laminarin, fucoidan, and porphyrin. Overall, a high chemodiversity of carbohydrate-active enzymes is considered beneficial to the diversification of nutritional modes, and as such likely conveys high adaptive capacity to oligotrophic marine environments, new hosts and substrates (Janusz et al. 2017; Roik et al. 2022).

6.4.5 Highly Diverse and Flexible Specialised Metabolism

Fungi produce a large diversity of secondary, or 'specialised' metabolites: structurally unique bioactive compounds act as key molecules in fungal chemical communication, defence and competition, facilitating interactions with their hosts and other microbes (Kusari et al. 2012; Keller 2019). These molecules may govern a range of intra- and interspecific interactions, conveying collective benefits or shaping microbial community compositions through antagonistic interactions (Hogan 2006; Schoenian et al. 2011). Quorum sensing (QS) is a major example for chemical mediation of collective behaviour in microbial populations. This term describes a concerted, density-dependent cell-to-cell signalling process in bacteria and fungi (Hogan 2006; Barriuso et al. 2018; Miller and Bassler 2001; Tian et al. 2021; Reading and Sperandio 2006). Unicellular microorganisms produce QS molecules, which accumulate in the environment during population growth. Once the population density exceeds a critical threshold, the QS molecules induce a coordinated population-wide response such as virulence and pathogenesis, secondary metabolite production, enzyme secretion, sporulation, or morphological differentiation (Barriuso et al. 2018). Specialised or secondary metabolites also exhibit diverse bioactivities with multiple putative ecological roles and are the main reason why fungi have long attracted natural product chemists and bioprospection efforts (Keller 2019). Specific examples for the considerable chemodiversity of coral-associated fungi are provided below in Sect. 6.5. One of the particularities about fungi, which have often been referred in the literature as "secondary metabolites factories" (Nielsen and Nielsen 2017), is their capacity to shift their metabolism and produce completely different arrays of specialised metabolites in different environmental conditions by "switching on and off" of biosynthetic gene clusters that code for specific metabolite production (Mózsik et al. 2022; Rokas et al. 2020).

6.4.6 Rapid Genome Adaptation

The average fungal genome is small and dynamic, but can range from a size of around 2 Mb (comparative to some bacterial genomes) in the parasitic Microsporidia to around 2 Gb in the Pucciniales (i.e., comparable to the size of the human genome) (Stajich 2017) harbouring between 1800 and 35,000 protein-coding genes in the Microsporidia and Agaricomycotina, respectively (Stajich 2017). Fungal genomes have a high capacity for rapid adaptive evolution (Feurtey and Stukenbrock 2018; Roik et al. 2022). Among other characteristics not further discussed here (but see Roik et al. 2022), fungal genomes can harbour a range of transposable elements (Hess et al. 2014; Miyauchi et al. 2020; Gluck-Thaler et al. 2022; Feurtey et al. 2023; Bucknell et al. 2024; Bucknell and McDonald 2023) contributing between 1 and 90% to the fungal genome in the plant pathogens *Fusarium graminearum* and *Blumeria graminis, respectively* (Cuomo et al. 2007; Frantzeskakis et al. 2018). High abundances of transposable elements are in concordance with weakened genomic defences against transposable element insertion in some fungi (Feurtey et al. 2023). In addition, many fungal genomes bear the hallmarks of interspecific gene exchange through hybridization of frequent horizontal and viral gene transfer (HGT, VGT) (Bian et al. 2020; Wang et al. 2021b; Gluck-Thaler et al. 2022; Kuroki et al. 2023). While the latter two mechanisms remain poorly studied in fungi, HGT was proposed to occur between distant terrestrial fungi (Soanes and Richards 2014) and non-fungal organisms including their hosts. Such gene exchange via hybridization, HGT and VGT may give rise to novel adaptive traits and adaptive capacity with new ecological niches and hosts (Soanes and Richards 2014; Feurtey and Stukenbrock 2018). HGT and VGT may also mediate switches from pathogenic to non-pathogenic lifestyles (Zhou et al. 2021) or may result in extension of a fungal recipient's physiological repertoire through the reception of complete or partial gene clusters, and thereby novel metabolic pathways (Feurtey and Stukenbrock 2018). These mechanisms have primarily been studied in pathogenic terrestrial lineages (Friesen et al. 2006; Menardo et al. 2016).

6.4.7 Functional Gene Content

Microbial genomes in oligotrophic marine systems typically harbour a diverse repertoire of different transporters and catalysts that function at alkaline pH and under the ionic stress that is associated with life in the ocean (Moran et al. 2004; Bonugli-Santos et al. 2015). The marine model yeast *Dendryphiella hansenii* harbours a genome characterised by high numbers of carbon and nitrogen transporters due to gene duplications and an overall reduction of non-coding DNA and gene lengths, resulting in increased coding densities compared to terrestrial yeasts (Lépingle et al. 2000). Such gene duplications were previously interpreted to reflect adaptation to demanding environments that select for the retention of duplicated genes even when changes in associated protein activities may be minor (Dujon et al. 2004). It remains to be determined whether such gene duplications are overall more common in yeasts and/or fungi living in the marine environment.

6.4.8 Gene Expression Dynamics

Changes in gene expression during certain stages in the fungal life cycle, for instance during conidiation (i.e., the formation of conidiophores from vegetative hyphae), may result in phenotypic variation in response to changing environments. A prominent example is the change of transcriptional activity and conidial mRNA, protein and metabolite content in *Aspergillus nidulans*, *A. fumigatus*, and *Talaromyces marneffei* until the release and subsequent dormancy of conidia (Wang et al. 2021a). Conidia that were pre-conditioned under different environmental conditions synthesise and store transcripts in response to the prevalent environmental condition, and, following germination, they exhibit environment-specific responses. The pre-conditioning of developing conidiophores hence affects stress responses, antifungal resistance capacity, mycotoxin and secondary metabolite production, and virulence (Wang et al. 2021a). This 'environmental priming' mechanism and flexibility of their conidiophores may in part explain how aquatic fungi have evolved from members of terrestrial lineages to survive in the new aquatic surroundings for instance through successful attachment to submerged substrates, displacement, and 'sticky drifting' (Grossart et al. 2019).

6.5 Chemical Diversity and Biological Activities of Reef-Associated Fungi

Coral-derived fungi produce diverse and structurally unique specialised metabolites, such as terpenoids, alkaloids, peptides and aromatic or phenolic compounds, which display cytotoxic, anti-microbial (anti-bacterial, anti-fungal and anti-viral), antioxidant and anti-fouling activities (Fig. 6.4). Such compounds are hypothesised to play a role in controlling the development of pathogenic microorganisms within

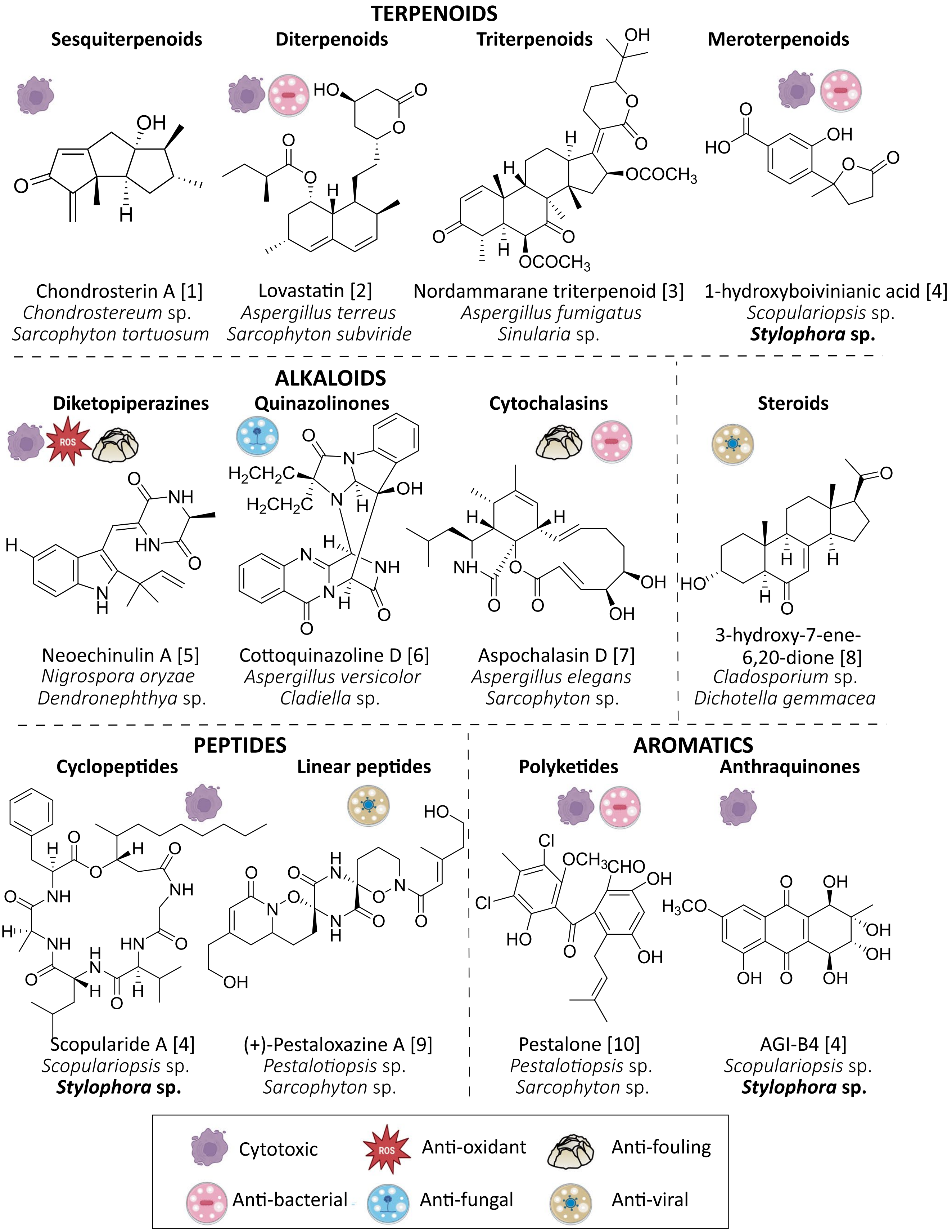

Fig 6.4 Types of compounds (top line) isolated from fungi derived from different hard and soft corals, showcasing their structural variability and associated bioactivities (symbol). The fungal strain (middle) from which the compound was isolated and the original holobiont (bottom line) are indicated. Hard coral holobionts are displayed in bold. Numbers refer to studies in which compounds were originally characterised. [1] Liu et al. (2013), [2] Liu et al. (2018), [3] Afiyatullov et al. (2012), [4] Elnaggar et al. (2017), [5] Sun et al. (2014), [6] Zhuang et al. (2011), [7] Zheng et al. (2013), [8] Yu et al. (2018), [9] Jia et al. (2015), [10] Wei et al. (2013)

terrestrial host organisms, most prominently in plant hosts (Elhamouly et al. 2022), but their role within corals or other reef invertebrates is mostly unexplored. Coral-derived fungi also produce a high variety of steroids, some of which have been shown to display powerful anti-viral activities (Yu et al. 2018) (Fig. 6.4). A recent study showed that a coral associated bacterium of the genus *Endozoicomonas* metabolises steroid hormones, which it may potentially provide to the coral host under heat stress (Ochsenkühn et al. 2023). Plant-associated fungi are known to produce phytohormones and phytohormones mimics, giving fungi the ability to interact with, and sometimes hijack, plant hormonal pathways (Ochsenkühn et al. 2023; Han and Kahmann 2019). Given the ability of coral-associated fungi to produce a wide range of steroids, some of which being hormone precursors (e.g., pregnanes (Yu et al. 2018)), it might be possible that they may interact with or disrupt host hormonal pathways with consequences for downstream functions, such as growth, reproduction and inter-kingdom communication. Recently, *Penicillium* fungi were suggested to provide several hydroxylated fatty acids to different gorgonian species (Sikorskaya et al. 2022) in a similar manner to Symbiodiniaceae (Papina et al. 2003). In corals, various fatty acids are involved in key functions in cell membrane structure, energy storage and coral fitness, and the specific roles of these fungi-derived fatty acids in the coral holobiont remain to be elucidated (Bergé and Barnathan 2005).

Although a better knowledge of the chemodiversity of coral-associated fungi will be critical for our understanding of the metabolic potential of coral-associated fungi, most of the current research on this topic stems from bioprospecting efforts that aim for natural product discovery (Chen et al. 2022a; Liu et al. 2021). In such studies, fungal strains are often isolated from the marine holobionts, e.g. corals, and cultured under different environmental conditions. Then metabolites are isolated and their structures characterised (Smith et al. 2019). Novel natural product discovery pipelines involve genome mining approaches, where the genomes of sequenced organisms are used to identify either novel Biosynthetic Gene Clusters (BGCs) or novel metabolites within known BGCs (Costantini 2021). As mentioned in Sect. 6.4.5, fungi have the ability to change their metabolite production depending on the environmental conditions by "switching on and off" of biosynthetic gene clusters that code for metabolite production (Mózsik et al. 2022; Rokas et al. 2020). Therefore, knowledge about metabolites and their bioactivities, acquired through culture-dependent or genomic approaches, may not be readily transferable to ecological questions, as many of the metabolites identified in vitro might not be produced in vivo, i.e., in the coral holobiont. As such, in vivo research, where fungal metabolic capabilities (i.e., metabolite production and bioactivities) are studied under biologically-relevant conditions (i.e., within their holobiont), is paramount to elucidate the roles of fungal secondary metabolites for marine invertebrate hosts and the ecosystem. For a better understanding of metabolic interactions between fungi and their host, We must also consider their potentially diverse interactions with other microorganisms, since many host responses to fungi may be mediated by prokaryotes or other microbes, as observed in various mammalian hosts (Romani et al. 2015).

Finally, given the high flexibility of fungal metabolism, it is plausible to expect that changing environmental conditions related to global change may affect enzymatic activities and hence the production of specialised metabolites within the coral holobionts. Such environmental control and its consequences for coral health still remain in the dark. However, assuming fungi may be a source of key metabolites (e.g., fatty acids, steroids, vitamins) for corals under stressful conditions such as elevated temperatures, this could potentially increase the hosts' resistance to stress, as it has been proposed for coral-prokaryotic symbiont associations (e.g., coral-*Endozoicomonas* symbiosis, (Ochsenkühn et al. 2023; Pogoreutz et al. 2022). However, changing environmental conditions could also alter the production of toxic metabolites and modify microbe-microbe interactions in unknown ways, and may potentially also trigger opportunism and pathogenicity in associated fungi, as proposed for sea fan aspergillosis (C. Toledo-Hernández et al. 2008; Rypien et al. 2008).

In summary, fungal functions within coral holobionts are likely tightly related to the metabolites produced, as these will govern interactions within the holobiont. It is therefore critical to understand the chemodiversity and biological activities of the metabolites produced by host-associated fungi (e.g., within the coral holobiont and on the reef) to gain novel insights on the relevance of fungi for the entire coral reef ecosystem (Roik et al. 2022).

6.6 Proposed Functions of Fungi and FLOs on the Coral Reef

6.6.1 Coral Reef Biogeochemical Cycling

Considering our knowledge from other marine systems about the extensive battery of exoenzymes of fungi, resulting in extraordinary substrate range and affinity (Newell 1996; Zhao et al. 2014a; Hagestad et al. 2021; Thomas et al. 2022), fungi on the reef might very well contribute to the remineralization of recalcitrant organic matter, and thereby the cycling of carbon, nitrogen, phosphorus, and sulphur (Gutiérrez et al. 2011, 2020). While no such studies are available yet, these are among the most pressing questions in coral reef microbial ecology and biogeochemistry (Newell 1996; Zhao et al. 2014b; Hagestad et al. 2021; Thomas et al. 2022). Marine fungi and FLOs may thereby contribute to the saprobic mobilisation of organic carbon on the reef. Such saprobic mobilisation could include the remineralization of recalcitrant high molecular weight detritus (Gutiérrez et al. 2011, 2020; Thomas et al. 2022), but of course parasitic routes as described from freshwater systems cannot be ruled out (Klawonn et al. 2021) (Fig. 6.5).

Nitrogen cycling by fungi and FLOs is be of particular importance, as nitrogen is a major limiting element in the oligotrophic tropical ocean and healthy photosymbiotic coral holobionts are typically nitrogen-limited (Cardini et al. 2015; Nils Rädecker et al. 2015; Pogoreutz et al. 2017). Reef-associated fungi may potentially satisfy much of their nitrogen requirements for growth from the degradation of algal-derived photosynthates (Dring 1992) and of recalcitrant polymeric compounds including chitin (Kirchner 1995; Tang et al. 2006), the most abundant polysaccharide in the ocean (Keller-Costa et al. 2022). Due to the presence of significant fungal rather than bacterial denitrification in redox-dynamic coastal sediments in the German Wadden Sea (Wankel et al. 2017), it was proposed that fungi may also be drivers of nitrogen remineralization in reef sediments (although marked differences in the (a)biotic properties of both ecosystems must be taken into account (Roik et al. 2022;) (Fig. 6.5). Indeed, reef sediments are characterised by significant microbial turnover of organic matter such as recalcitrant coral mucus aggregates, which feeds benthic and pelagic productivity on coral reefs through the release of limiting inorganic nitrogen and phosphorus (Wild et al. 2004; Wild et al. 2005). Hence, within coral holobionts, fungal metabolisms may help prevent the loss of nitrogen from the holobiont (Rädecker et al. 2015). Indeed, holobiont-studies have identified different fungal genes and gene expression associated with nitrogen metabolism and transport. This includes the metabolism of nucleic acids, amines, and cellular nitrogen compounds, and of enzymes involved in urea, glutamate, glutamine, and ammonification metabolisms (Wegley et al. 2007; Amend et al. 2012). As such, it was previously proposed that fungal nitrogen metabolism and cycling likely accounts in part for the high levels of inorganic nitrogen in the interstitial pore water of coral skeletons (Le Campion-Alsumard et al. 1995) (Fig. 6.5).

Phosphorus and sulphur cycling properties of fungi and FLOs in the ocean remain largely unknown, and have not been investigated on the reef. In general, the macronutrient phosphorus limits oceanic bacterial (Van Wambeke et al. 2002) and photosymbiotic activity (Wiedenmann et al. 2012; Nils Rädecker et al. 2015) and governs pelagic marine thraustochytrid distribution and biomass across space and time (Bongiorni and Dini 2002). Fungal involvement in phosphorus cycling could be driven by their remineralization activity in reef sediments and coral skeletons (Risk and Muller 1983; Wild et al. 2004) or by primary mineral weath-

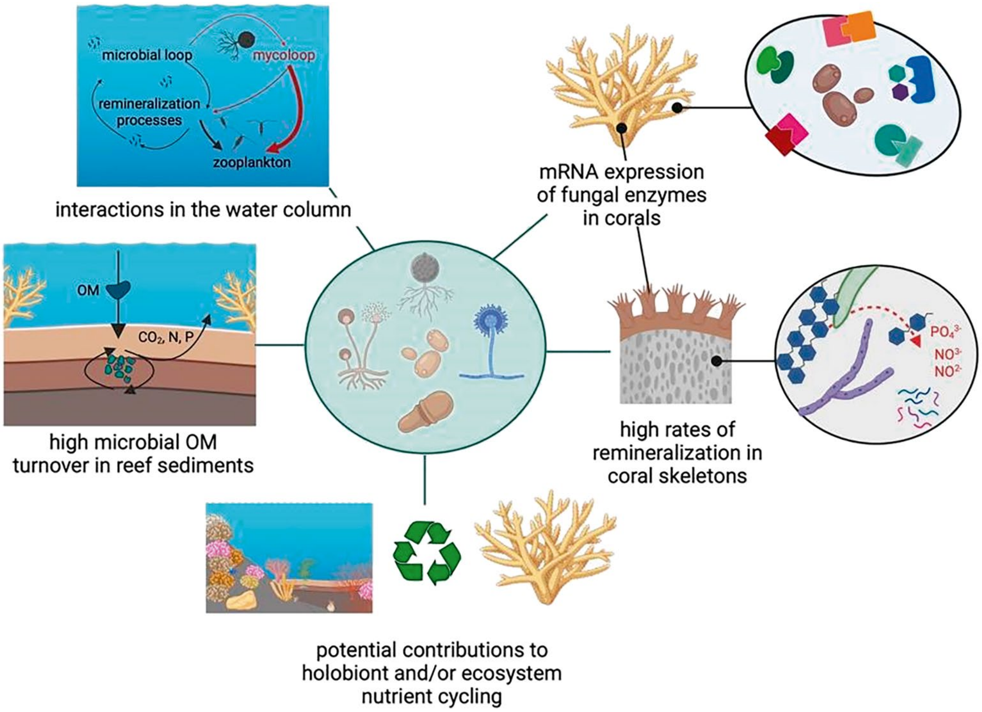

Fig. 6.5 Overview of known and proposed functions and interactions of aquatic and/or reef-associated fungi with regard to mineralization and biogeochemical cycling. While fungal activities and contributions to the reef food web remain to be determined, their considerable chemodiversity and versatility of exoenzymes may permit fungi to contribute to reef biogeochemical cycles. Such contributions may differ between pelagic vs. benthic environments, on the ecosystem vs. (sub) holobiont level. Figure created in BioRender

ering as observed in terrestrial fungi (Landeweert et al. 2001), which could in part explain the high phosphate levels in coral skeleton pore water (Risk and Muller 1983). Such fungal phosphorus provisioning could help alleviate nutrient limitation for other endolithic organisms or potentially even the host tissues at the millimetre to centimetre scale (Roik et al. 2022).

Finally, some marine fungal isolates are capable of metabolising different (in)organic sulphur compounds (including sulfides and methanethiol). Fungi could hence be active contributors to coral reef sulphur cycling (Wainwright 1989; Faison et al. 1991; Phae and Shoda 1991; Bacic and Yoch 1998). For instance, *Fusarium lateritium* are known to degrade DSMP from algae and the salt-marsh grass *Spartina alterniflora* (Bacic and Yoch 1998), while the coral pathogen *Aspergillus sydowii* actively expresses DMSP lyase dddP in the presence of DMSP (Kirkwood et al. 2010). Fungal DMSP transformations may be ecologically relevant in the coral holobiont., considering the abundance of DMSP in the coral holobiont and the ecological relevance of this compatible solute in osmoregulation and antioxidant responses (Raina et al. 2009; Santoro et al. 2021).

In conclusion, marine fungi may be ecologically important players in the complex process that includes the (coral) holobionts and reef biogeochemical cycling. Likely, the respective importance and magnitude of individual pathways will vary across spatial and temporal scales and levels of biological organisation (Roik et al. 2022). Novel experimental and computational approaches will certainly help elucidate fungal biogeochemical cycling on coral reefs from the cellular to the ecosystem level.

6.6.2 Reef Framework Eroders

Fungi and FLOs have been reported from different coral reef environments including the reef framework, where they penetrate the reef substrate, which includes the solid carbonate reef rock and calcareous skeletons of living corals (Pernice et al. 2019; Ricci et al. 2019). Several fungal genera have been isolated from coral skeletons among which *Fusarium*, *Cladosporium*, *Aspergillus*, *Penicillium*, and *Xylaria* sp. were the most prominent isolates (Yarden et al. 2007; Kendrick et al. 1982; Hosen et al. 2023). While the ecological roles of these endolithic fungal communities in benthic holobiont and ecosystem functioning (Pernice et al. 2019; Ricci et al. 2019) are not well understood, there are some indications that these organisms may drive reef bioerosion processes, and as such may contribute towards the total carbonate budget (Perry and Harborne 2016; Roik et al. 2018).

Inside the skeleton of living corals, endolithic fungi likely contribute to nutrient generation in the pore water by organic matter decomposition (Risk and Muller 1983; Priess et al. 2000), and are considered bioeroders, parasites, or opportunists (Yarden et al. 2007; Gleason et al. 2017). Histological studies showed fungal “attacks” on the endolithic green algae *Ostreobium*. At the same time, hyphae of endolithic fungi attempt the penetration of living coral tissues from the skeleton below (Bentis et al. 2000). These attacks are seemingly countered by the coral through the continuous accretion of aragonite, resulting in characteristic perl-like depositions or ‘capsules’ around the hyphae (Bentis et al. 2000). Interestingly, isolates of the basidiomycete *Cryptococcus* from coral selectively prolong the short-term survival of skeletogenic (but not other) coral cell types in vitro, which could reflect a stimulation of coral defence reactions in response to the fungal intruder (Domart-Coulon et al. 2004). However, potentially beneficial effects of *Cryptococcus* on skeletogenic coral cells have not been ruled out yet. The possibility remains that these opportunistic saprophytic fungi may be able to bypass compromised immune defences of vulnerable coral hosts, which is supported by observations of e.g. the high amounts of septate fungal hyphae contained in the bleached tissues of fire coral *Millepora complanata* after a marine high temperature anomaly (Strake et al. 1988).

The potential metabolic interactions of endolithic fungi with other members of the coral holobiont remain poorly understood. Roik et al. (2022) have proposed that endolithic fungi may divert photosynthate from coral-associated algae, as recently described in pelagic freshwater diatom–chytrid pathosystems (Gleason et al. 2008; Klawonn et al. 2021). While this remains hypothetical, such interactions could become problematic during times of prolonged environmental stress, when organic carbon translocation from endolithic algal communities to the coral host may become physiologically relevant (Fine and Loya 2002). Indeed, the depletion of alternative endolithic carbon supplies by parasitic fungi could further exacerbate the health of the already starving and stressed coral host. However, it remains to be determined whether this proposed interaction indeed occurs, and whether it is ecologically relevant in healthy and stressed corals (Roik et al. 2022).

6.6.3 Opportunists, Parasites, and Pathogens

Some of the first reports of fungi on coral reefs focussed on an emerging marine disease associated with gorgonian octocorals, commonly known as sea fans (Alker et al. 2001; Smith and Weil 2004; Geiser et al. 1998). *Aspergillus sydowii* has been isolated from diseased corals and transfection experiments established its association with a disease-like phenotype, subsequently named ‘aspergillosis’ (Alker et al. 2001; Smith et al. 1996). The cosmopolitan genus *Aspergillus* grows on a broad range of substrates, is very well known for its association with infectious disease in animals and humans (Seyedmousavi 2019) and for its mycotoxins which can cause allergic reactions in humans (Tanimoto et al. 2015) or spoil crops (*A. flavus* and *parasiticus* (Gourama and Bullerman 1995)). *A. sydowii* occurs on both, healthy and diseased corals alike and most recent evidence indicates that *A. sydowii* may not be the (sole) cause of 'aspergillosis' (C. Toledo-Hernández et al. 2008; Toledo-Hernández et al. 2013; Becker et al. 2023). Monitoring of outbreaks and in vitro observations of the cultured fungus indicate that increased temperature is a likely cause of the disease-like phenotype (Mullen et al. 2004; Ward et al. 2007; Alker et al. 2001). As such, two scenarios are possible: (1) Pathogenicity of *A. sydowii* is induced by increased temperature, resulting in the fungus attacking its host or (2) the fungus is an opportunistic invader of the coral tissues due to declining host immune defences under thermally challenging conditions (Rypien et al. 2008; Toledo-Hernández et al. 2008). The fungal invaders may rely on chemical defences and cues to engage with (or attack) other members of the holobiont. Metabolites of *A. sydowii* can negatively affect the photosynthetic efficiency of coral-associated algal endosymbionts (Hayashi et al. 2016). Also, virulent *A. sydowii* strains are attracted by and metabolise DMSP, a compatible solute abundantly produced by the Symbiodiniaceae, corals, and associated bacteria, and suspected to be central to holobiont functioning and coral health (Kirkwood et al. 2010; Lawson et al. 2019). Finally, a recent pathological study of diseased gorgonian tissues presenting with aspergillosis (Leverette et al. 2008) were not only populated by hyphae, but also algal and protist cells, cyanobacteria and labyrinthulomycetes, suggesting mixed opportunistic colonisation (Becker et al. 2023).

Importantly, different species of *Aspergillus* are associated with marine holobionts, where their functions remain unknown. Strains of *A. hiratsukae* are frequently isolated from gorgonians and soft corals where they inhabit the mucus or tissues, while maintaining an apparently neutral relationship with their host. Metabolites of these coral-derived *Aspergillus* strains however are potent bioactive agents in various contexts (tumor inhibition, anti-oxidatant activity, α-glucosidase inhibitory activity), with many expressing strong antibacterial activity (Zeng et al. 2022; Chen et al. 2022b). As such, these broad bioactivities suggest these mucus-associated *Aspergillus* species may be implicated in host health, such as in structuring host mucus-associated microbial communities, as proposed for some aquatic host-associated bacteria (Pogoreutz et al. 2019; Bowman 2007; Lowrey et al. 2015).

The question whether fungi like *Aspergillus* are opportunistic (terrestrial) intruders or native to marine environments still remains to be clarified. Many of these fungal species, including groups of known opportunistic pathogens, may be airborne and associated with Saharan dust. Indeed, dust deposition events can carry terrestrial particles containing fungal cells or spores (e.g., *Aspergillus*, *Thielavia*, *Penicillium*, *Chaetomium strumarium*, *Periconia*, and *Cladosporium sphaerospermum*) from Africa to as far as the Caribbean, where sea fan aspergillosis was originally described (Ramírez-Camejo et al. 2022).

Another fungal coral reef disease affects the functional group of crustose coralline algae (CCA, or corallines). CCAs are ecologically important reef-builders which help stabilise the three-dimensional reef structure (Perry and Hepburn 2008), contribute to the reef carbonate budget, and act as attractive reef substrate for coral larvae settlement (Price 2010). The Coralline Fungal Disease (CFD) was first described in American Samoa (Littler and Littler 1998), where lesions on CCAs have been observed which led to mortality of affected CCA. The alga *Porolithon onkodes* has been documented as the major host for CFD throughout the tropical Pacific and Indian Oceans (Williams et al. 2018). Histopathology has identified a member of the Ustilaginomycotina, a fungal group that includes a number of terrestrial plant parasites, to be associated with the lesions. Sequencing suggested that fungi were closely related to *Malassezia restricta*. The fungus has been shown to infect CCA host surface tissues at a rate of up to 6.5 mm/day (Williams et al. 2014). Manipulative experiments further showed that elevated temperature favours the spread of the fungus, while lower seawater pH (i.e., ocean acidification scenarios) can minimise outbreaks (but does affect the calcification potential of the CCA host). Grazing fish are likely another environmental factor that contributes to reducing the impact of the fungi on their infected host algae (Neal et al. 2020). A recent study described the beneficial effect of the presence of grazing fish and reported the first observations of fish feeding on the fungal patches, which was associated with reduced disease progression on heavily grazed reefs (Neal et al. 2020).

FLOs being parasites and causing disease and disease-like phenotypes have been long known from a range of marine holobionts and marine ecosystems. In an attempt to identify the causative agent of multifocal purple spot (MFPS) disease, members of Labyrinthulomycota belonging to the family Thraustochytriidae were isolated from the Caribbean Sea fan *Gorgonia ventalina* (Burge et al. 2012). *Aplanochytrium* and *Thraustochytrium* were isolated from diseased and visibly healthy hosts respectively. However, the inoculation with either isolate did not induce MFPS disease. Similar to *A. sydowii*, it was suggested that Labyrinthulomycetes may be opportunistic pathogens in sea fans (Burge et al. 2012; Dennis et al. 2020). As such, further experimental study will be required to conclusively confirm or rule out pathogenicity of Thraustochytriidae in reef-associated Cnidaria (Bonacolta et al. 2023).

Labyrinthulomycetes are known parasites and/or opportunistic pathogens in seagrasses, clams, and nudibranchs (Ragone Calvo et al. 1998; Ragan et al. 2000; McLean and Porter 1982; Tan et al. 2021; Trevathan-Tackett et al. 2018).

Here, seagrass beds provide probably the most compelling case of outbreaks of emergent diseases associated with FLOs. Labyrinthulomycetes, Oomycetes and Phytomyxea are the most prominent groups considered "disease-forming organisms" on seagrasses (Sullivan et al. 2018). Among these, *Labyrinthula zosterae*, a marine and freshwater heterotrophic protist in the group of net slime molds, was linked to a massive die-off of *Zostera marina* meadows in the 1930s. *Labyrinthula zosterae* was isolated from affected seagrass blades and experimentally confirmed as the causative agent (Muehlstein 1992). In brief, *L. zosterae* penetrates seagrass blades, subsequently causing chloroplast and cell necrosis (Sullivan et al. 2018). However, members of *Labyrinthula* have since been found on healthy and diseased meadows alike (Brakel et al. 2014, 2019). Indeed, related species are known to decompose plant material in marine and coastal ecosystems—an ecosystem function which they perform in mixed communities of thraustochytrids and aplanochytrids (Tsui et al. 2009). Consequently and similar to the activity of saprobic fungi, invasion of blade tissues may be opportunistic behaviour. Recently, re-evaluations of the role of Labyrinthulomycetes in seagrass meadows take more holistic approaches, considering environmental and host factors, which has so far revealed a highly dynamic pathosystem governed by salinity, depth, and host population genetics as the main drivers of virulence (Jakobsson-Thor et al. 2018).

Many algal pathogens have been identified as members of the fungi or FLOs. Zoosporic fungi, i.e., Chytridiales, Blastocladiales, Rhizophydiales, and e.g. *Paraphysoderma sedebokerense* are most widely studied (Murúa et al. 2023). Chytrids for instance are cosmopolitan, known to infect brown, green, and red algae, diatoms, and photosynthetic alveolates (e.g., dinoflagellates) (Murúa et al. 2023). Many of these emerging pathogens have started affecting the billion-dollar valued seaweed markets in Asia, where they represent the fastest-growing aquaculture sector (FAO 2020). Oomycetes are well known for their ability to infect not only algae but also plants, invertebrates, and vertebrates, and their marine members are most closely related to terrestrial plant pathogens. Examples are *Pythium porphyrae* which causes red rot disease in the red alga *Porphyra* (laver seaweed) or the obligate intracellular *Eurychasma dicksonii*, an early-diverging branch of Oomycota, known to infect different brown algae (Klochkova et al. 2017; Murúa et al. 2023). Similarly, phytomyxids are increasingly raising attention, in part because of their capacity for broad cross-kingdom host compatibility, affecting plant, brown algae, diatom, and even oomycete hosts (Neuhauser et al. 2014).

These glimpses into the known diversity of marine fungi and FLOs and their mostly opportunistic interactions with marine hosts strongly suggest that many such interactions are yet to be discovered on coral reefs—some of which might help explain the current unknowns associated with disease, coral bleaching and even macro-community dynamics of coral reefs in the Anthropocene. We hence propose that investigations into the fungal and FLO groups on coral reefs should become a priority to better understand, forecast and prevent future emerging disease outbreaks driven by fungi and FLOs that might cause irreparable damage on coral reefs that will entail severe economic losses in the future.

6.6.4 Fungi as Mutualists and Potential Probiotics

To date, no known example of mutualistic or commensal fungi in corals exists. This may however be due to a systematic bias towards the study of opportunistic and pathogenic fungi, a trend also apparent in the study of human and crop diseases (Feurtey and Stukenbrock 2018). Considering the high biodiversity of tropical coral reefs, the challenges associated with the study of marine and coral-associated microbes in particular (Pogoreutz et al. 2020, 2022; Robbins et al. 2019), and in the face of recent discoveries of several new marine symbiotic relationships owing to recent technical advancements in other fields (Shao et al. 2020; Schvarcz et al. 2022; Zhang et al. 2021a; Mordret et al. 2016), Roik et al. (2022) have concluded that there is an appreciable potential for diverse, yet so far entirely overlooked mutualistic relationships of marine fungi with reef holobionts and microorganisms.

Mycorrhiza are a remarkable, mutualistic guild of terrestrial fungi, associating with most plant species and providing benefits to their host plants through the provisioning of nutrients and minerals, but also known to increase the survival of their host trees during cold winters and drought (Landeweert et al. 2001; Allsup et al. 2023). While no reef-associated mycorrhiza are known, mycorrhiza and endophytic fungi are assumed to account for a substantial proportion of nitrogen present in the decaying standing plant biomass at the land-ocean interface of salt marshes (Newell 1996; Otte and Landy 2006). Different species of seagrasses have been reported to harbour endophytic, i.e. internal tissue-colonising fungi in different parts of the plant, including the roots which can be dominated by dark septate endophytes (Borovec and Vohník 2018; Alva et al. 2002; Vohník et al. 2015). Their presence has been confirmed by various tools, including culturing, metagenomics, and histology (Seshagiri Raghukumar 2017). In kelp (red and brown algae), the filamentous endophytic fungi *Paradendryphiella* and *Dendryphiella* reportedly produce metabolites with strong antibacterial and anti-oomycete activity, suggesting a protective role of fungal endophytes on their seaweed hosts (Vallet et al. 2018). In corals, assessment of the functions and metabolic activities of fungal endophytes and surface-associated yeasts has mostly (but not exclusively) focused on their anti-microbial potential (Supaphon et al. 2013). Importantly, while non-opportunistic or even mutualistic endophytic, tissue-associated fungal associations in corals have yet to be described, their existence at this point cannot be conclusively ruled out.

Recent years have seen a significant rise in conceptual and experimental studies to identify beneficial coral-associated microbes and to develop effective probiotic treatments for the mitigation of the effects of global environmental change on reef-building corals. While the ecological roles of reef-associated fungi remain to be determined, we know that fungi in general harbour functional traits of potential significance for this challenging quest, including but not limited to their significant chemodiversity and bioactive potential (Peixoto et al. 2021; Roik et al. 2022). Mycorrhizal fungi can contribute to oxidative homeostasis in the plant holobiont (Nath et al. 2016; Huang et al. 2017), and oxidative stress has been proposed as a driver of stress responses in corals (Michael P. Lesser 1996; Lesser 1997). As such, antioxidant properties are a major trait of interest for probiotic applications currently being investigated in bacterial consortia and single isolates and first promising candidates tested for their potential to alleviate the detrimental effects of reactive oxygen species arising under environmental stress (Rosado et al. 2018; Dungan et al. 2021; Peixoto et al. 2017). This, for instance, encompasses the fungal production of mycosporine-like amino acids (MAA) that could offer UW protection, or the stimulation of the growth of skeletogenic cells in corals (Dunlap and Shick 1998; Domart-Coulon et al. 2004) among other putative protective functions as proposed in Peixoto et al. (2017, 2021). Further, one of the main drivers of coral bleaching is the disruption of nutrient cycling in the holobiont and starvation of the host (Rädecker et al. 2021; Rodrigues and Grottoli 2007). It was hence proposed that potential candidates for probiotic fungal strains could include organisms known to contribute to nutrient cycling, such as different functional groups of nitrogen cyclers, to help contribute towards nutritional homeostasis in the stressed holobiont (Roik et al. 2022). In addition, considering the enzymatic (Gostinčar and Gunde-Cimerman 2018; Massaccesi et al. 2002) and secondary metabolite diversity of fungi (Ritchie 2006; Xu et al. 2015) which may be of potential aid in the structuring and maintenance of coral-associated microbiomes, further investigation into the suitability of potential fungal probiotics for stress mitigation in corals is certainly warranted.

First experiments have already successfully tested fungal potential for chemical remediation in oil spell scenarios on coral reefs, compared to the activity of a commercial chemical dispersant (Silva et al. 2021). The authors included three oil-degrading fungal isolates, *Geotrichum* sp., *Rhodotorula mucilaginosa*, and *Penicillium citrinum*, obtained from corals, into a bacterial probiotic consortium. The authors found that the resulting experimental multi-domain consortium was efficient in degrading different oil fractions and did not negatively affect coral holobiont physiology, while the chemical dispersant exhibited more detrimental effects than the oil treatment itself, resulting in mortality, coral bleaching, tissue damage, and reduced quantum yield of the algal symbiont photosystem (Silva et al. 2021). This study highlights the importance for carefully controlled experiments as proof-of-concepts for the application of marine probiotics, and contributes to the growing body of literature underlining the importance of including aquatic fungi into concepts of environmental remediation and species conservation (Vatova et al. 2022).

6.7 The Challenge of (Marine) Fungal Community Characterization

In recent years, marine fungi have began to shift into the focus of high-throughput, next-generation sequencing efforts. Such efforts to understand the diversity and functions of marine fungi will be crucial not only to help elucidate their roles in the environment, but also from the perspective of bioprospection for pharmaceutical and biotechnological applications (Marchese et al. 2021; Li et al. 2014) (refer to Sect. 6.5). However, our knowledge of even very basic information about marine fungi, such as their community composition in marine holobionts or various substrates, and phylogenetic classification of fungi remains poor. Existing databases mainly contain sequences obtained from terrestrial fungi, which has resulted in an inadequate representation of marine genetic diversity (Rabbani et al. 2021). Nonetheless, the increased application of culture-independent approaches has expanded our understanding of fungal diversity and community complexity, highlighting their potential importance in marine ecosystems (Amend et al. 2019; Roik et al. 2022). However, the characterization of fungal communities remains challenging as techniques like PCR-based gene amplicon sequencing and metagenomics face limitations.

One challenge is specific to the assessment of fungal communities in complex holobionts. In metazoan hosts, including corals and other reef holobionts, sequencing of the 18S ribosomal rRNA gene (Schoch et al. 2012) and Internal transcribed spacer (ITS) regions (Bellemain et al. 2010) poses challenges as many commonly used universal primers tend to co-amplify the DNA of the eukaryotic host (Schoch et al. 2012; Cavalier-Smith et al. 2014; Taylor et al. 2016; Frau et al. 2019). Such co-amplification issues can lead to low sequencing coverage of host-associated fungi, and thereby an underestimation of their diversity (Amend et al. 2012; Bonthond et al. 2018; Góes-Neto et al. 2020; Paulino et al. 2020). Similarly, in environmental samples, fungal-specific primers tend to co-amplify zooplankton and other invertebrates commonly found in the marine environment (Amend et al. 2019), which can affect fungal sequencing coverage (Amend et al. 2019; Bonthond et al. 2018; Góes-Neto et al. 2020). Alternatively, phylum-specific primers designed to avoid the amplification of non-target DNA achieved greater proportions of fungal reads (up to 22%) in coral samples (Bonthond et al. 2018). This highlights the critical importance of primer design and selection in fungal community studies (Wiesmann et al. 2022; Taylor et al. 2016; Walters et al. 2016; Tedersoo et al. 2022; Bokulich and Mills 2013). Recent studies have shown the superior performance of several anti-metazoan primer pairs, which can effectively enhance microeukaryotic sequencing coverage across various animal hosts (Wiesmann et al. 2022; Del Campo et al. 2019; Bass and Del Campo 2020), by targeting specific metazoan sequence signatures, for instance, downstream of the V4 region in the 18S rRNA gene largely absent in most other eukaryotes. This approach outperforms the conventional universal primer approach, which predominantly amplifies the host's 18S rRNA gene (Del Campo et al. 2019; Bass and Del Campo 2020; Minardi et al. 2022). Another approach to minimise co-amplification of host DNA regions includes the use of blocking primers designed to overlap primer binding sites of co-amplifiable organisms (e.g. coral host, algal symbionts, or other abundant microeukaryotes) and prevent elongation through a 3′-end modification (Banos et al. 2018; Clerissi et al. 2018). The implementation of blocking primers has been successful in increasing fungal read coverage, with up to 80% of reads originating from fungi in environmental and coral samples (Banos et al. 2018; Clerissi et al. 2018). In addition, implementing blocking peptide nucleic acid (PNA) clamps has proven effective to reduce co-amplification of host DNA (e.g. plants) (Azadnia et al. 2023) up to 65% (Taerum et al. 2020; Fitzpatrick et al. 2018), resulting in successfully increasing the number of fungal reads (Azadnia et al. 2023; Borodušķe et al. 2023; Viotti et al. 2024) In some cases, this has also resulted in an increase in the detected fungal diversity, without introducing bias on the composition of eukaryotic groups (Taerum et al. 2020; Moccia et al. 2020). Nonetheless, primer selection and targeted regions of the ITS region and 18S rRNA gene are critical aspects to consider, as they influence the efficacy of blocking amplification by the PNA clamps (Borodušķe et al. 2023; Viotti et al. 2024). On another note, it will be interesting to combine primers targeting different markers, and to develop primer pairs targeting protein-encoding regions and functional genes rather than conventional marker genes to characterise specific functional groups of fungi (for details, see Sect. 6.8 below).

For metagenomic studies, additional considerations have to be taken into account. For instance, pervasive amounts of host-derived DNA typically results in high sequencing coverage of host-associated reads compared to microbial reads. To increase the proportion of microbial reads in complex holobiont samples, different measures can be taken. For instance, commercial kits such as host-zero kits are now available which deplete host-derived DNA and a selective lysis method for host cells that does not lyse fungal cells, followed by the enzymatic removal of host DNA (Rabbani et al. 2021; Bonthond et al. 2018). This method has proven successful, as it increased the proportion of fungal sequences to more than 75% of the overall sequences in coral samples (Rabbani et al. 2021; Bonthond et al. 2018). Furthermore, improving the sampling design of fungal surveys can be achieved through targeted sample collection and methodological refinements, especially when dealing with complex host model organisms. One such approach is to selectively target specific host structures or compartments, for instance mucus, tissue, and skeleton in corals, during sample collection (Rabbani et al. 2021; Cárdenas et al. 2022). Such targeted sampling can help minimise contamination (e.g. from host tissues) during pre-DNA extraction steps to increase sequencing coverage.

Finally, recent advances in imaging techniques may aid our exploration of fungi in holobionts, specifically allowing for in situ localization and identification. These techniques employ fluorescent staining and taxon-specific DNA probes to visualise fungal cells in complex environmental samples (Roik et al. 2022; Amend et al. 2019; Gladfelter et al. 2019). Another exciting recent advancement for the field is the development of 'secondary metabolite FISH' (SecMet-FISH), which leverages the conserved nature of BGCs, hence targeting the genetic basis of non-ribosomal peptide and polyketide biosynthesis (Buijs et al. 2024). Importantly, such imaging approaches could be combined with microfluidics and flow cytometry to isolate single cells from environmental samples and implementing single cell-genomics (Ahrendt et al. 2018) and culture-dependent work, including functional interrogation: the study of metabolic interactions, whole genome sequencing, and the examination of expressed genes (Sieracki et al. 2019; Swan et al. 2011; Ahrendt et al. 2018). Ideally, multi-omics approaches should be combined with traditional classification approaches such as microscopy and histological methods focusing on cell and spore morphologies to further elucidate fungal identities (Dayarathne 2020). Such an integrated approach will enable us to comprehensively expand our inventory of fungal diversity and to assess their putative roles in marine ecosystems and holobionts across space, time, and levels of biological organisation (Gladfelter et al. 2019). In summary, such coordinated efforts will help improve the phylogenetic classification and respective databases of fungal diversity one sequence at a time. Ultimately, these efforts will also help us advance our understanding of coral microbial interactions by facilitating manipulative studies for mechanistic insight and concept validation. As such, improving isolation and culturing success of marine and reef-derived fungi (for instance, implementing novel methods to uncover microbial dark matter (Schultz et al. 2022; Nichols et al. 2010; Zhang et al. 2024)) will become increasingly important to identify their likely diverse functions and interactions in reef holobionts (Roik et al. 2022).

6.8 Outlook

Moving forward in the field of coral- and coral reef-associated fungal communities will require substantial efforts from various angles. This includes improvements of current metabarcoding protocols and databases, developments of new laboratory and analytical pipelines to increase the coverage of fungal sequences in complex environmental samples, as well as the establishment of best practices for functional work at different levels of biological organisations.

1. Gene markers such as the 18S rRNA remain a viable choice for mycobiome studies, but are prone to substantial cross-amplification with host DNA (Scholz et al. 2016) study of fungi in marine holobionts or other biomass-rich samples. Combinations of different markers such as ITS, SSU and LSU rRNA, along with protein-encoding regions could be employed to counteract cross-amplification issues and significantly increase specific sequence yields (Tekpinar and Kalmer 2019). Important improvements in metabarcoding protocols besides the development of group-specific primers, blocking or anti-metazoan primers to reduce the amount of host- and Symbiodiniaceae-derived sequences (Clerissi et al. 2018; Del Campo et al. 2019), specific markers for fungal functional genes should be developed, focusing on potentially important metabolic pathways such as different CAZymes or nitrogen cycling pathways (Roik et al. 2022). Finally, long read- and hybrid sequencing applications for different marker genes or metagenomes could be leveraged (Lücking et al. 2020; Furneaux et al. 2021). At the same time, database optimization and expansion will be critical for the interpretation of marine fungal diversity (Martorelli et al. 2020).
2. Improved isolation techniques will be crucial for functional research on coral reef fungi. Here, Kjer and colleagues have provided an excellent and highly detailed step-by-step methodological framework for the isolation of marine-derived fungi and their secondary metabolites (Kjer et al. 2010). Adaptation of such protocols to accommodate diverse marine animal, plant, or algae hosts and substrates, along with custom modifications for specific fungal functional groups to expand to a greater diversity of microbial growth media (Raghukumar 2017) and conditions may greatly help maximise the yield and diversity of culturable marine fungi. In addition, customised microfluidics platforms could potentially be harnessed to accommodate a diversity of microeukaryotes of different shapes, including filamentous and branched fungi (Millet et al. 2019), and in combination with high-throughput microbial culturing help increase isolation success. The use of novel co-culturing, or synthetic small scale environments in microcosms (Raina et al. 2022) could further aid in the discovery of novel marine fungi, metabolites, and biotic interactions (Roik et al. 2022).
3. Another important point is the establishment of fungi model organisms for functional interrogation. While some well-established yeast lab model systems for genetics and molecular work such as *Saccharomyces cerevisiae* or *Hortaea werneckii* exist, no marine or reef-associated fungal lab models have yet been established. The establishment of laboratory model systems requires coordinated and dedicated community efforts, but the knowledge gain it promises will be worth such efforts. As such, one focus of future work should be on the phenotypic and genomic characterisation of new reef-associated fungal isolates to provide a basis for further screening for suitable model fungi, and, importantly, functional work to elucidate complex interactions within holobionts and ecosystems. At the same time, considering the potentially enormous fungal functional diversity present on coral reefs, it may be impossible or inadequate to select only one candidate species or strain. While further isolation and characterization efforts are required, a potential candidate for the study of coral fungal disease-like phenotypes could be the notorious *Aspergillus sydowii*. Future studies will certainly uncover more metabolically diverse fungi to be shortlisted for the ranking of suitable coral (reef) fungal model organisms.

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Part III

Emergent Complexity

The Coral Holobiont

7

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Abstract

Here we emphasize the crucial role of microbial communities in the biology of coral hosts, presenting the concept of the coral holobiont as the main unit interacting with other organisms and the environment. This concept offers profound insights into the symbiotic relationships that define coral health and resilience, guiding future research and conservation efforts.

Keywords

Microbiome, Bacteria, Environmental adaptation, Holobiont, Metaorganism

7.1 The Holobiont as the Phenotypic Unit

Previous chapters described the importance of different groups of microbes {i.e., Symbiodiniacea (Chapter 2), bacteria (Chapter 3), endolithic algae (Chapter 4), viruses (Chapter 5), and other microbial entities (Chapter 6)} that can contribute to the coral host's biology. Overall, the development, growth, and health of organisms are influenced by the sum of the associated microbial groups independently discussed in this book, which will collectively compose complex microbial communities affecting the physiology of their hosts (Santoro et al. 2025; McFall-Ngai et al. 2013; Selosse et al. 2014; Foster et al. 2017; Ziegler et al. 2019). Therefore, individual phenotypes are the result of complex interactions between hosts and their associated microorganisms, which generated the 'meta-organism' and 'holobiont' concepts (Bordenstein and Theis 2015).

The term "holobiont" was first introduced in 1991 by Lynn Margulis (Foster et al. 2017), and initially referred to a simple biological entity involving a host and a single inherited symbiont. This term was later expanded to define a host and its associated microbiome (i.e.,the collection of microorganisms interacting among themselves and with their host, ranging from mutualistic to parasitic interactions) (Rohwer et al. 2002; Berg et al. 2020), and is the most commonly used term in coral-related sciences.

Inter-kingdom associations benefit the holobiont with evolutionary, immunological, anatomical, and physiological traits (Gilbert et al. 2012; Bordenstein and Theis 2015; Simon et al. 2019) that exceed the sum of its parts. Research focused on the holobiont as a phenotypic unit have spread over a vast spectrum of disciplines exploring the evolution and adaptability of holobionts (Zilber-Rosenberg and Rosenberg 2008; Theis et al. 2016; Morris 2018; Baedke et al. 2020), including human medicine, functional ecology, and terrestrial and marine sciences (Foster et al. 2017).

Overall, the functions provided by host-microbiome interactions seem to be largely similar across different hosts, whereby crucial microbial mechanisms, such as nutrient cycling and production of key metabolites, protection against pathogens, and mitigation of toxic compounds contribute to the holobiont's health, resilience, growth, development, and reproduction (Peixoto et al. 2021a). Interestingly, plants and animals share a single-cell organism ancestor (Knoll 2011), and many functions and adaptations have evolved convergently following the emergence of multicellularity (Foster et al. 2017). For example, host epithelial interfaces with the associated microbiome have convergently evolved in humans, corals, and plants (Foster et al. 2017). Vertebrates, invertebrates, and plants develop specific epithelial surfaces where a complex microbiota can grow (Foster et al. 2017). In corals, for example, insights from the last decade(s) have revealed that released nutrients, antimicrobial and signaling

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compounds, key metabolites, and mucus or mucilage may help the respective hosts to interact with or control its associated microbiome (Ferrier-Pagès et al. 1998; Wild et al. 2004; Yellowlees et al. 2008; Rädecker et al. 2015; Wiedenmann et al. 2023; Falkowski et al. 1984; Muscatine and Porter 1977; de Goeij et al. 2013; Pawlik and McMurray 2020), whereas the symbiotic microbiome can translocate nutrients from their end, and offer protection from pathogens through antimicrobial release and other mechanisms (Roediger 1980; Rypien et al. 2010; Philippot et al. 2013; Buffie et al. 2015; Hacquard et al. 2015).

Furthermore, symbiotic single-cell algae (Symbiodiniaceae) (LaJeunesse et al. 2018) that thrive within the coral tissues harvest light energy via photosynthesis, with energy-rich photosynthates being shared with the cnidarian host (Muscatine and Porter 1977). In turn, these symbionts benefit from the hosts' waste products, which are essential to perform photosynthesis. This symbiotic relationship is crucial for the coral holobiont but also for the wider reef ecosystem as the symbiotic algae produces energy in excess that is translocated to the reef ecosystem in the form of coral mucus (Wild et al. 2004). The lack of these symbiotic partners, induced by stressors, such as increasing water temperatures or increasing nutrient availability, represent major impacts on the holobiont heath (Hughes and Connell 1999; Rädecker et al. 2021).

In a nutshell, most living organisms rely on their associated microbiomes, which quickly respond to environmental changes. Under stress, these microbiomes can shift from a beneficial, mutualistic assemblage towards a pathogenic state (i.e., referred to as dysbiosis—(Peixoto and Voolstra 2023). This dynamic relationship between hosts and their microbiomes is fundamental to understanding, predicting, and ensuring holobiont health. Consequently, studying such host-microbiome interactions has become essential for the development of tools that can quickly restore the health and function of holobionts (Costello et al. 2012; Lozupone et al. 2012; Buffie et al. 2015; Coyte et al. 2015; Peixoto and Voolstra 2023; Rosado et al. 2023; Voolstra et al. 2024).

7.2 Diving Deeper Into the Interconnected Partnerships Within the Coral Holobiont

Specifically focusing on corals, their associated microorganisms play specific and essential roles in the holobiont homeostasis and adaptability capacity, including key mechanisms such as carbon uptake, nitrogen and sulfur cycling, antimicrobial production, and the mitigation of excessive amounts of reactive oxygen species (ROS) (Peixoto et al. 2017; Rosado et al. 2019; Santoro et al. 2021; Peixoto et al. 2021b; Rosado et al. 2023; Doering et al. 2023; Raimundo et al. 2024)—Fig. 7.1). Even though technical advances (e.g., genomics, transcriptomics, metabolomics, proteomics) and polyphasic approaches (i.e., the use of several culture-dependent and -independent tools—e.g. (Santoro et al. 2021; Cardoso et al. 2024) have allowed a more holistic view on the coral holobiont, most of the data relies on studies exploring isolated portions of the holobiont (i.e. coral-Symbiodiniaceae or coral-bacteria). The interactions between the different groups within the holobiont are usually overlooked, despite their potential importance.

For example, the interaction between free-living phytoplankton (including Symbiodiniaceae in its free-living stage) and their associated bacteria seems to be a key aspect contributing to their nutrition and survival (Jeong et al. 2012; Frommlet et al. 2015; Lawson et al. 2018), while in hospite Symbiodiniaceae are also commonly associated with bacteria (Maire et al. 2021; Hill et al. 2024). Free-living-phytoplankton exude metabolites that attract and support the growth of other microorganisms via chemotaxis (Seymour et al. 2017; Frommlet et al. 2015; Maire et al. 2021), creating an enriched zone around themselves (Amin et al. 2012; Seymour et al. 2017; Shibl et al. 2020), similar to the rhizosphere effect observed in plant roots (Raaijmakers et al. 2009; Philippot et al. 2013). Known as the "phycosphere" (Bell and Mitchell 1972) (Fig. 7.1), this physical interface between algae and their surrounding environment might selectively promote associations with other microeukaryotes, bacteria, archaea, and viruses (Garrido et al. 2021). In addition to the production of molecules involved in inter-organism signaling, antioxidative responses and photosynthates that can attract and be consumed by associated bacteria also drive microbial associations (Lawson et al. 2020). Dinoflagellates are known to produce Dimethylsulfoniopropionate (DMSP), an organic sulfur compound that can act as a chemoattractant for bacteria (Seymour et al. 2010; Raina et al. 2010), as well as antioxidants (Sunda et al. 2002) and osmolytes (Stefels 2000). DMSP-degrading bacteria, known for their beneficial role in mitigating the effects of coral bleaching within the coral holobiont (Santoro et al. 2021), are commonly found in association with dinoflagellates and corals (Miller and Belas 2006; Varaljay et al. 2012), which highlights the importance of microbe-microbe (i.e., Symbiodiniaceae-bacterial) interactions in structuring the holobiont microbiome and health.

In addition to the co-existence of multiple microorganisms and interactions, the coral spatial compartmentalization, including their skeleton, tissue, mucus, and cellular compartmentalization (i.e., the symbiosome) (Wakefield and Kempf 2001), adds even more layers of complexity (Fig. 7.1). Each of these spatial compartments encompasses different microbial assemblages and functional repertoires (Sweet et al. 2011; Apprill et al. 2016; Pernice et al. 2020). The

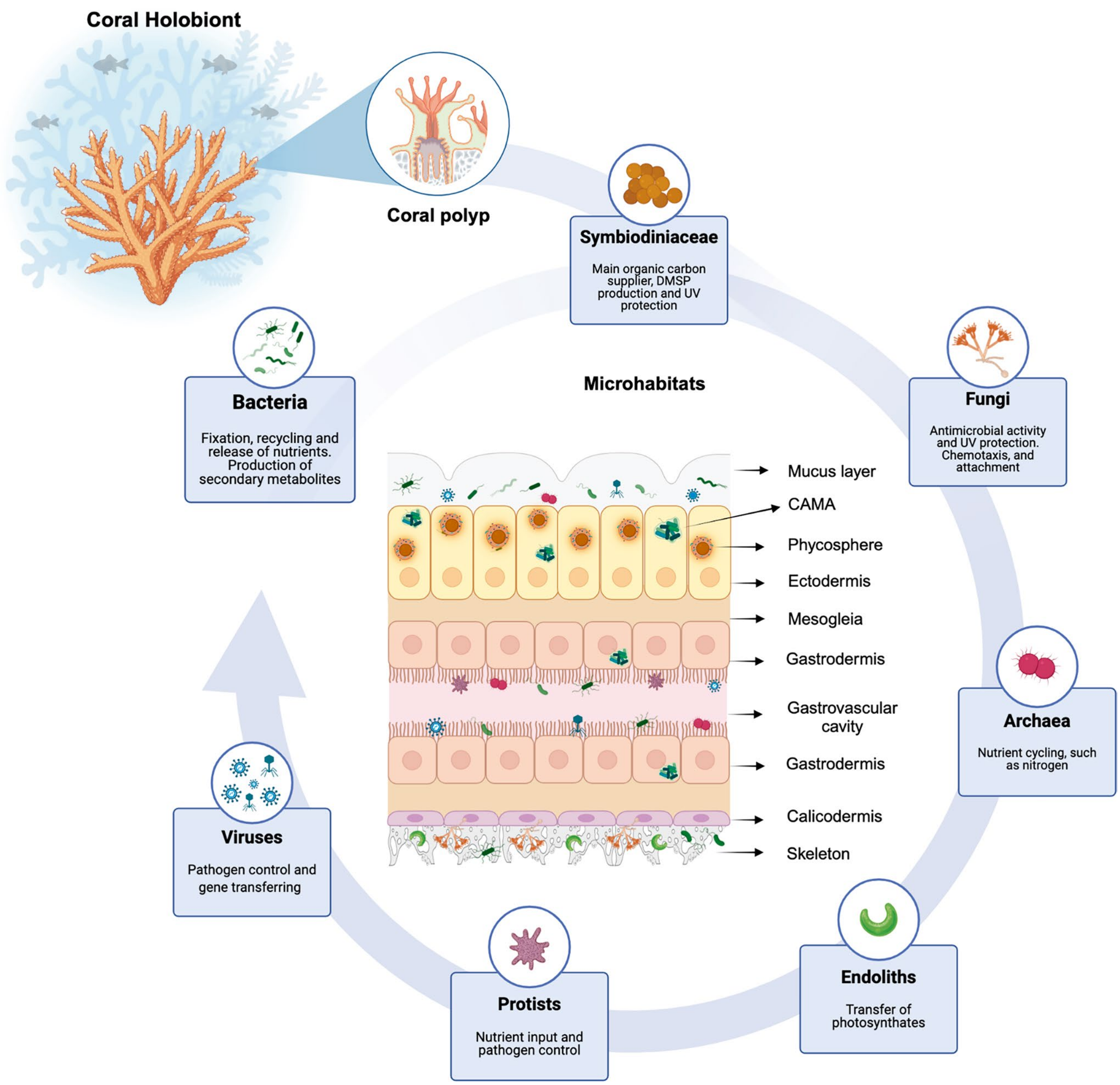

Fig. 7.1 Coral microhabitats and their associated microorganisms within the holobiont. The size of the Symbiodiniaceae and microbes has been modified for illustration purposes. Figure created with BioRender.com

mucus layer, for example, consists of a nutrient-rich microhabitat with high amounts of carbohydrates, lipids, and proteins (Brown and Bythell 2005; C. Wild et al. 2010; Nakajima et al. 2015), harboring a high concentration of transient and associated bacteria (Marchioro et al. 2020; Garren and Azam 2010; Paul et al. 1986; Weiler et al. 2018). The mucus layer acts as a physical barrier to protect the coral against desiccation and trapping particulates, and its composition, viscosity, and thickness also seem to respond to environmental stress, consequently modulating the bacterial community structure (Lee et al. 2016). In turn, the mucus-associated bacterial community can also prevent pathogen colonization by antibiotic production or by modifying the nutritive and physical characteristics of this microhabitat (Krediet et al. 2013).

Within the tissue layers (ectodermis and gastrodermis), symbiosomes containing the key photosynthetic algal symbiont (Fitt and Trench 1983; Davy et al. 2012) and aggregates of bacteria (termed coral-associated microbial aggregates, or CAMAs) can be found (Ainsworth et al. 2006; Ainsworth and Hoegh-Guldberg 2009; Wada et al. 2019; Work and Aeby 2014; Maire et al. 2023; Bayer et al. 2013; Neave et al. 2017b). Recent co-localization studies revealed that *Simkania*

(Clamydiota) co-inhibits and/or forms adjacent CAMAs with *Endozoicomonas* within *Pocillopora acuta* tissues (Maire et al. 2023). Bacteria from these genera seem to play important roles within holobiont fitness by synthesizing multiple amino acids, B vitamins, and antioxidants, as well as participating in carbon cycling and prey digestion (Maire et al. 2023, 2024). Validated coral symbiotic beneficial bacteria, such as *Halomonas* sp. and *Cobetia* sp., used as probiotics, have also been found within the coral tissue, although not yet searched within CAMAs (Cardoso et al. 2024).

The complex and porous calcium carbonate structure of the coral skeleton represents the vastest internal volume of corals, providing a great micro-environment for the most diverse microbial community within the coral holobiont, which includes endolithic algae, fungi, heterotrophic bacteria, and other boring eukaryotes (Pernice et al. 2020); Tribollet 2008; Verbruggen and Tribollet 2011; Ricci et al. 2019).

Defining the microbiome core and diversity of each micro-habitat within the holobiont and how they are potentially connected is essential for elucidating their contribution to the holobiont, which will be further explored in Chapter 8. Dynamic associations are likely common in the mucus layer due to its interaction with the surrounding seawater. Conversely, microbial associations with the coral skeleton and tissue may be more stable and consist of an important target for studies on coral resilience and microbiome manipulation (van de Water et al. 2018; Pollock et al. 2018; Robbins et al. 2019; Cárdenas et al. 2022).

Although the role, distribution, and function of the majority of prokaryotes in the coral holobiont still need to be further explored, especially considering that geographic location is one of the forces driving the coral microbiome assemblage (which will also be discussed in more detail in Chapter 8), different taxonomic groups are consistently associated with corals, regardless of the location and flexibility of the coral's host microbiome (Voolstra and Ziegler 2020). Among these taxonomic groups, some are overall related to healthy corals, such as *Roseobacter* sp. (K. H. Sharp et al. 2015), *Halmononas* sp., *Cobetia* sp. and *Pseudoalteromonas* sp. (Rosado et al. 2019; Cardoso et al. 2024), while others are found more frequently in unhealthy corals, such as some species of the genera *Vibrio* (Ushijima et al. 2012) and *Rhodobacter* (Mouchka et al. 2010). Additionally, some genera, such as *Endozoicomonas* (Neave et al. 2017a), can be found associated with both healthy and unhealthy corals (Pogoreutz and Ziegler 2024).

A comprehensive review of the distribution of bacterial taxa in different coral species throughout the Red Sea (Delgadillo-Ordoñez et al. 2022) indicate that the families *Vibrionaceae* and *Rhodobacteraceae* are the most prevalent among the coral species examined, representing approximately 85% of the coral samples. This observation suggests that these families are ubiquitous, at least within the microbiome of Red Sea corals. Although both families are often associated with unhealthy corals, evidence also suggests their beneficial roles in the holobiont, including nutrient cycling (Dryselius et al. 2007), degradation of polycyclic aromatic hydrocarbons, and production of antibiotic substances (Thompson et al. 2004; Raimundo et al. 2018; Miura et al. 2019; Luo et al. 2021). Other abundant families found in the Red Sea are *Flavobacteriaceae*, *Pseudomonadaceae*, *Endozoicomonadaceae,* and *Halomonadaceae*, all of which have previously been described as having potentially beneficial properties for the corals (Howard et al. 2011; Peixoto et al. 2021b; Neave et al. 2016; Neave et al. 2017a).

Vibrio is the most abundant genus among the various corals in the coral microbiome database (Huggett and Apprill 2019), which integrates sequences of coral-associated microbes, followed by the genera *Endozoicomonas* and *Ruegeria* (*Rhodobacteraceae* family). This further demonstrates that, despite the correlation between the *Vibrionaceae* and *Rhodobacteraceae* families and unhealthy corals observed in several studies, they are also prevalent in healthy corals. This may be due to a diversity of representatives within these groups, including beneficial and harmful species, in addition to a defining feature of opportunistic pathogens, which, in a benign non-stressful environment, may be present but are non-pathogenic. However, when the environment undergoes a shift that is detrimental to the holobiont, such as an increase in ocean temperatures, both families may overgrow and become pathogenic.

7.3 A Dynamic Phenotypic Unit

The coral holobiont adjusts to environmental changes and stressors as a single phenotypic unit, and such response can happen in different ways, either through adaptation or acclimatization (Savolainen et al. 2013; Fordyce 2006). In addition to such host-centered mechanisms, the associated microbes represent a more plastic and dynamic entity to adjust to changing environments (Voolstra and Ziegler 2020). Such plasticity may play a pivotal role in holobiont health (Santoro et al. 2025; Webster and Reusch 2017; Baldassarre et al. 2022; Zhou et al. 2021; Osman et al. 2023; Vohsen et al. 2020) and can be divided into three scales of environmental adaptation. These include the relative increase or decrease of certain bacterial associates (Ziegler et al. 2019), the association with novel bacteria (from the environment) (Webster and Reusch 2017) at the species and strain level (Ansorge et al. 2019), or the acquisition of novel genes for instance via horizontal gene transfer (HGT) (Voolstra and Ziegler 2020).

Symbiodiniaceae, also characterized by their shorter generation time (compared to the host) and elevated genetic and functional diversity, can expand the ecological niche of corals, enabling them to flourish in diverse habitats. Consequently, these symbionts can potentially serve as a source of epigenetic modifications to promote holobiont phenotype changes in response to emerging environmental stressors (reviewed in (Gilbert et al. 2010). Notably, coral hosts can switch (i.e., acquire new symbionts from the environment) or shuffle symbiont (i.e., replace dominant species with new ones) populations in response to changing environments (Webster and Reusch, 2017). In both approaches, selective pressures may promote the acquisition of heat-tolerant symbionts by the host. Quigley and colleagues (Quigley et al. 2022) demonstrated that *Acropora* spp. restructured its Symbiodiniaceae community towards more heat-tolerant species after a bleaching event in the Great Barrier Reef. Exposure to marine heatwaves revealed that corals have a dynamic symbiont composition that enables survival after prolonged and recurrent heat waves (Claar et al. 2020).

7.4 Ontogeny of Holobiont Assemblage: Acquisition of Symbionts in Coral Early Life Stages

Not only adult corals rely on their associated microbiome, but such key interactions are also important during their early life stages. As sessile animals, corals have evolved and developed specific strategies to reproduce and maintain their population diversity. Corals can propagate asexually through processes that include budding polyps (Kramarsky-Winter and Loya 1996), colony fragmentation (Highsmith 1982), and polyp expulsion - “polyp bailout” (Schweinsberg et al. 2021), in which genetically identical clones (ramets) are generated. In order to guarantee their genetic diversity, corals reproduce sexually as broadcast spawners or brooders. In broadcast spawning corals, eggs and sperm are released into the water column for external fertilization (Harrison 2011; Bouwmeester et al. 2016). This strategy often leads to synchronous spawning events, typically triggered by environmental cues such as water temperature and lunar cycles (Guest et al. 2005; Baird et al. 2009; Keith et al. 2016). In brooding corals, egg-sperm fertilization (either auto-fertilization or sperms that travel between colonies) occurs internally, where larvae are brooded and then maturely released to the water column (Harrison 2011). Coral reproductive strategies and species-specific seem to influence the mode of symbiont acquisition in gametes and/or larvae “at birth”, which seem to occur through: 1, vertical transmission (parent colonies transmit their symbionts to their offspring); 2, horizontal transmission (acquisition of symbionts from the surrounding environment); 3, mixed mode of acquisition (when both vertical and horizontal modes are observed). While vertical transmission may allow corals to maintain specific mutualistic partners without depending on a potentially unpredictable symbiont source, horizontal acquisition offers the advantage of uptaking symbionts from the environmental pool that may provide a local ecological benefit (Byler et al. 2013; Hartmann et al. 2017).

The acquisition of Symbiodiniaceae occurs during the early life stages of the coral development and seems to vary according to the reproductive strategy. Vertical transmission of Symbiodiniaceae is mainly found in brooding coral (Baird et al. 2009; Cumbo et al. 2012), which is hypothesized to generate a lower diversity but high fidelity symbiotic interaction (Barneah et al. 2004; Thornhill et al. 2006; Stat et al. 2008). Thus, the reproductive strategy and the mode of Symbiodiniaceae transmission seems to also correlate with the associated bacterial community (Padilla-Gamiño et al. 2012; Quigley et al. 2017).

Given the importance of bacteria to holobiont biology and their presence in virtually all coral’s compartments, exploring its colonization and symbiotic establishment during early stages is also crucial to gaining insights into the interactions between the coral host and the associated microbiome. Studies using fluorescence in-situ hybridization (FISH) targeting the 16S ribosomal RNA (rRNA) genes indicated the presence of bacteria cells in brooded larvae (Sharp et al. 2012; Maire et al. 2024), suggesting bacterial vertical transmission in corals presenting this reproductive strategy. However, different brooder *Pocillopora* species seem to inherit a small number of taxa from the parent colony as well as acquire bacteria from the surrounding environment (Epstein et al. 2019; Damjanovic et al. 2020). Bacteria forming CAMAs were also observed to be vertically transmitted from parent colonies to their larval offspring and were hypothesized to provide beneficial functions to their coral host, such as the production of vitamin B and antioxidants and cycling of carbon and amino acids (Maire et al. 2024).

Bacteria were also detected through molecular screenings in gametes and planula larvae of broadcast spawning corals (Leite et al. 2017; Zhou et al. 2017; Bernasconi et al. 2019; Damjanovic et al. 2020), although no bacterial cells were yet detected in these gametes using microscopy (Leite et al. 2017; Damjanovic et al. 2020). These results suggest either immediate horizontal transmission (Ceh et al. 2013) or, more likely, vertical transmission through the bundle and/or coral mucus in broadcast spawning corals (Leite et al. 2017; Bernasconi et al. 2019; Damjanovic et al. 2020). However, limitations of FISH technique due to sample processing might have impacted the detection of bacterial cells in these gametes. For example, the crucial fixation of samples on paraformaldehyde leads to the loss of the mucus layer, which consequently impacts the visualization of bacteria associated with it (Johansson and Hansson 2012; Chiu et al. 2012). Additionally, sectioning small materials such oocytes was demonstrated to be challenging and might lead to missing vertically transmitted bacteria that are likely to be found in low concentrations (Damjanovic et al. 2020). Further studies are therefore necessary to understand if these bacteria are transmitted directly to the gametes or immediately during or after spawning. Furthermore, hypothetically, changes in the microbiome may also promote epigenetic changes in the coral host (Barno et al. 2021), which could be transmitted to the offspring even without the transferring of members of the microbiome.

Understanding the adaptive strategies performed by each biological component of the holobiont and the integrated responses of the coral holobiont as a whole can offer key targets to harness and restore their natural adaptability capacities (Peixoto et al. 2017; van Oppen and Blackall 2019; Li et al. 2023), for example through the use of coral microbiome stewardship (i.e., targeted microbiome management through microbial therapies or environmental management aiming at its restoration or rehabilitation) (Peixoto et al. 2022). Such microbial-based approaches can mitigate a range of impacts (Peixoto et al. 2024), including thermal stress, disease, and poor water quality, and will be further explored in Chapter 13.

7.5 Conclusions

This chapter highlights the holobiont as a unit encompassing the host and their associated microbiome. This perspective underscores the complexity of the interactions within the holobiont, where the combined expression of both host and microbial genes determines the phenotype and adaptability of the organism.

The diversity within coral holobionts is compartmentalized across different microhabitats, such as the mucus, the tissue layer, and the calcium carbonate skeleton. Each compartment hosts distinct microbial assemblages that contribute uniquely to the holobiont's overall function and health, collectively exhibiting differential adaptive strategies. Coral holobionts may also transmit microbial partners to their offspring, which could potentially transfer adaptive traits across generations.

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Complexity of the Coral Microbiome Assembly

8

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Abstract

The coral holobiont, comprising corals and their associated microbiomes, is a complex entity critical to reef health and ecosystem function. The composition of these microbial assemblages varies across coral species, coral compartments (e.g., skeleton, tissue, and mucus), coral genotypes, and environments. At the same time, certain bacteria are repeatedly and predictively associated with specific coral species, suggesting that coral microbiomes consist of transient, resident, and core bacterial associates. The composition and assembly of these distinct communities are a consequence of coral host-related and environmental factors. A good example of this interplay is the correlation between different host genotypes and their associated microbial assemblages, and how the referred holobiont responds to environmental conditions and change. At large, the extent to which microbiomes can change with the environment varies among coral host species, a concept termed microbiome flexibility. Knowledge about the dependence of specific coral species on their microbiomes, the ability to change microbial association in different environments, and the extent of functional redundancy between host genotypes are essential for predicting coral responses to environmental change and developing effective microbiome-centered conservation strategies, such as probiotic therapy.

Keywords

Microbiome assembly · Coral compartments · Core bacterial associates · Microbiome composition · Host genotype · Environmental factors · Microbiome flexibility · Coral-associated bacteria

8.1 The Complexity of the Coral Holobiont

Corals are home to diverse microbial communities, including microeukaryotes, prokaryotes, and viruses. The coral host, along with these consortia of microbes, is referred to as the coral holobiont (Rohwer et al. 2002; Rosenberg et al. 2007; Bourne et al. 2016). While the most routinely studied coral symbionts are dinoflagellate algae of the family Symbiodiniaceae (LaJeunesse et al. 2018) and bacteria (Voolstra et al. 2024; van Oppen and Blackall 2019; Bourne et al. 2016), corals also associate with other microbiome members that are less explored, including archaea, viruses, fungi, algae, and other microeukaryotes (Bonacolta et al. 2023; Roik et al. 2022; Mohamed et al. 2023). Members of the dinoflagellate family Symbiodiniaceae provide the coral host with most of its energy needs through photosynthetically derived organic matter (Bourne et al. 2016; Muscatine and Porter 1977) in exchange for access to inorganic carbon generated from respiration (Muscatine and Weis 1992; Falkowski et al. 1984; Cunning et al. 2017). This reciprocal exchange of metabolites in the coral-Symbiodiniaceae symbiosis is considered the engine of the holobiont that enables the deposition of calcium carbonate to construct coral skeletons and is the foundation of coral reefs (Pogoreutz et al. 2020).

While Symbiodiniaceae inhabit the gastrodermis layer within specific host-derived membrane structures called symbiosomes (Davy et al. 2012; Mohamed et al. 2016; Rosset et al. 2021), derived from phagosomes, bacteria can establish niches in all coral microhabitats, including the sur-

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face mucus layer (SML), tissue layers, the gastric cavity, and the skeleton (Neave et al. 2017; Pollock et al. 2018; van Oppen and Blackall 2019; Pernice et al. 2020; Sweet et al. 2011). Coral-associated bacteria are thought to support the physiology and resilience of corals through various mechanisms (Bourne et al. 2016; Ziegler et al. 2019; Voolstra and Ziegler 2020; Meunier et al. 2022; Ziegler et al. 2017). These mechanisms include nutrient recycling (Rädecker et al. 2015; Gardner et al. 2022; Rädecker et al. 2021a, b), B vitamin provision (Pogoreutz et al. 2022; Hochart et al. 2023), and the production of antimicrobials (Ushijima et al. 2023; Raina et al. 2016). These mechanisms are thought to help mitigate the effects of thermal stress (Peixoto et al. 2017; Santoro et al. 2021; Ziegler et al. 2017), and the provided functions are critical for the overall fitness of the coral holobiont that may play an important role in the coral response to climate change (Voolstra et al. 2024). Thus, understanding the coral-Symbiodiniaceae-bacteria tripartite symbiosis is key to unraveling the function of the coral holobiont and how corals will respond to climate change. Admittedly, many other microbes exist that associate with the coral holobiont in a more or less known manner, many of which are deemed functionally important, which will be discussed in other places in this book (Voolstra et al. 2021).

Over the past decade, an emphasis on the bacterial component of the microbiome has resulted in a wealth of 16S ribosomal RNA gene amplicon sequencing data from coral surveys. Despite several methodological limitations, such as amplification bias, choice of variable region, and difficulty in detecting low-abundance taxa, amplicon sequencing has proven invaluable in providing insights into coral microbiomes primarily because of its cost-effectiveness, enabling the incorporation of large sample numbers in coral surveys to achieve novel insight beyond the realm of corals (Galand et al. 2023).

8.2 The Concept of a Coral Core Microbiome

The widespread use of amplicon sequencing enables determining the presence of a putative core microbiome associated with corals across host genotypes and geography (Shade and Handelsman 2012; Lloyd-Price et al. 2016; Neu et al. 2021; Neave et al. 2017; Hochart et al. 2023). Identifying core members of the microbiome could pinpoint core pathways and metabolic functions underlying host-microbe interactions (Martínez-Álvaro et al. 2021; Lee et al. 2023). This approach has been applied to various eukaryotic host-associated microbiomes such as ants, plants, and humans (Sharon et al. 2022; Toju et al. 2018; Wu et al. 2023). The core microbiome framework in corals is still in its infancy (Sweet and Bulling 2017) but generally revolves around the microbiome being comprised of **(1)** a transient community assumed to be associated with the mucus that is mainly environmentally controlled (Ziegler et al. 2017, 2019; Roder et al. 2015), **(2)** a resident community that is selected by a particular coral host/genotype (Dubé et al. 2021; Buitrago-López et al. 2023), and **(3)** a core community that is universal or near-universal across multiple coral species irrespective of genotype or environment (Hernandez-Agreda et al. 2018; Hochart et al. 2023; Neave et al. 2017). This classification of the coral microbiome is consistent with observations from other marine hosts (Osman and Weinnig 2022). Notably, whether a certain bacterium is part of the core microbiome or not does not necessarily provide insight into its relative 'importance' in a given environment or for a given host genotype, as the very concept of core microbiome (universal presence) counters such notion (Bang et al. 2018). While understanding the importance of the most consistently present microbes to the biology of coral species or populations is crucial, they might be less informative with regard to understanding the contribution of bacteria to the changing environments of a particular coral species or population. For instance, if we are to pinpoint bacterial candidates for survival in an extreme environment, characterization of those microbes that are only present in this environment but not in a more benign environment is informative, whereas the conserved core microbiome between both environments might prove uninformative in this instance (arguably, these bacteria may be essential for the coral species, but not for survival in a specific environment) (Camp et al. 2020; Ziegler et al. 2017, 2019; Voolstra and Ziegler 2020). Additionally, functional redundancy, where different taxa perform the same functional role, complicates the notion of functional loss caused by the absence of certain taxa (Dubé et al. 2021; Louca et al. 2018; Cárdenas et al. 2022).

8.3 Coral Microbiome Composition Across Life Stages and Coral Compartments

Although some bacteria can be transmitted vertically from parent to offspring (Leite et al. 2017; Damjanovic et al. 2020b; Maire et al. 2024), corals are thought to majoritively acquire bacteria from the surrounding environment (Damjanovic et al. 2020a, b; Damjanovic et al. 2020). Initially the bacterial diversity is comparatively low during early developmental stages but increases with progress to the larval stages (Damjanovic et al. 2020b; Sharp et al. 2010). During the course of coral colony growth, bacterial diversity progressively increases as larvae are exposed to diverse bacterial communities in the water. This is followed by a stepwise elimination process that selectively recruits specific bacterial associates through competition and exclusion, culminating in the formation of stable associations maintained by a combination of host and bacterial factors, in a process known as winnowing (Voolstra et al. 2024; Nyholm and McFall-Ngai 2004; Damjanovic et al. 2020b; Chan et al. 2019). In adult corals, the microbiome varies between coral compartments. Bacterial taxa from the coral holobiont as a whole are markedly distinct from those found in coral polyps, the skeleton, the gastrodermis, or the symbiosomes (Apprill et al. 2016; Maire et al. 2023, 2024; Garren and Azam 2010), suggesting that different coral compartments harbor specific bacteria and that homogenizing whole host samples fail to resolve these differences. Samples acquired from 32 coral species from 21 sites spanning 17 degrees of latitude across Australia showed an anatomically partitioned microbiome distinct in the mucus, tissue, and skeleton (Pollock et al. 2018). These microhabitats are characterized by distinct biochemical signatures, which select for or enable the survival of specific microbial communities (Cárdenas et al. 2022; Engelen et al. 2018; Sweet et al. 2011).

8.4 Coral Microbiomes Are Shaped by the Interplay Between Host Genotype and Environmental Factors

Evidence strongly indicates that corals harbor host-specific microbiomes that vary in a species-specific manner across different environments (Ziegler et al 2019; van Oppen et al. 2018; Buitrago-López et al. 2023; Dubé et al. 2021; Kriefall et al. 2022). Studies analyzing natural coral populations with genetic markers (e.g., RAD-Seq, microsatellites) consistently show that while host genotype significantly influences microbiome assemblage, environmental conditions exert a stronger influence on microbiome composition compared to coral host genetics (Fig. 8.1). An exemplary study by Dubé et al. (2021) analyzed bacterial communities in fire coral clones across environments using twelve polymorphic microsatellite loci. Their experimental design effectively differentiated the contributions of host genotypes and environmental factors by comparing microbiome differences between host genotypes in the same environment and those of host clones in different environments. While reef habitat had a predominant influence on overall microbiome composition, the authors identified that members of the bacterial classes Alpha- and Gammaproteobacteria, Actinobacteria, and Spirochaetes were indicator taxa for host genotypes. Similarly, van Oppen et al. (2018) used 2,268 SNPs using RAD-Seq to identify coral genotypes of *Pocillopora damicornis* in reef flat and slope habitats at two locations on Heron Island in the southern Great Barrier Reef. The associated prokaryotic communities had a strong differentiation across environments, mainly driven by the abundance of the bacterial genera *Endozoicomonas* and *Brevibacterium.* Additional variation driven by coral genetic structure was evident at a smaller degree (van Oppen et al. 2018). In another study, Kriefall et al. (2022) used 2b-RAD sequencing to identify 3,594 SNPs in a highly connected *Acropora hyacinthus* population in French Polynesia. They found that reef zones were the main factor structuring microbial communities, but also reported correlations between coral genetic diversity and bacterial diversity only in corals located at the fore reef site (Kriefall et al. 2022). More recently, Buitrago-López et al. (2023) used RAD-Seq to identify 35,208 SNPs in *Pocillopora verrucosa* and 25,318 SNPs in *Stylophora pistillata* that resulted in two and six genetic host clusters across the Red Sea, respectively. This study showed that coral microbiomes were strongly shaped by the environment, with northern reefs being overall less diverse in both species and some bacterial groups, including members of the *Kistimonas* (Endozoicomonadaceae) that made up a considerable proportion of the bacterial community in the southern regions. A smaller fraction of the microbial variation was attributed to host genetics, in which bacterial alpha diversity differed significantly between host genetic clusters. In addition, this study identified biomarkers predominantly represented by the bacterial families Flavobacteriaceae, Rhodobacteraceae, and Endozoicomonadaceae that strongly

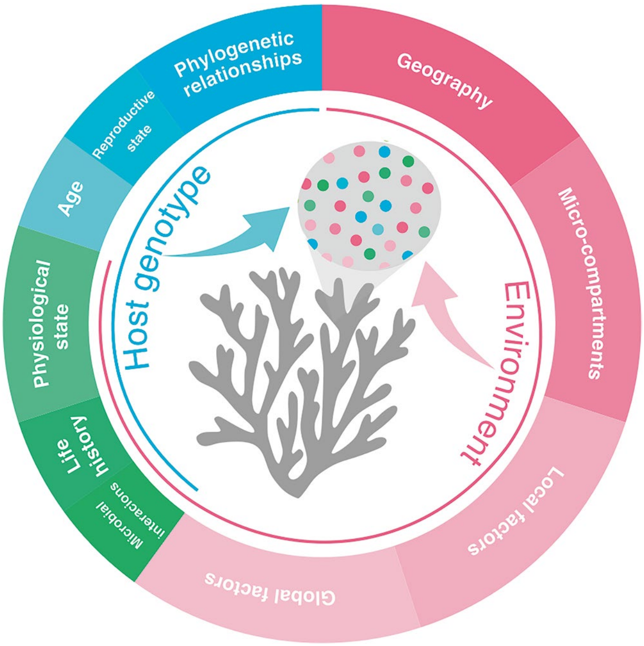

Fig. 8.1 Major factors influencing the composition of coral microbiomes. The coral host (blue) can affect its microbiome through its genetic makeup, physiological state, and life stage. Environmental factors (pink) can impact microbiomes in a host-specific manner, operating across scales from coral micro-habitats to global conditions. The interplay between host genotype and environmental conditions (green) synergistically shapes the microbiome by affecting coral physiology, ecological dynamics, and microbial interactions

correlated with host genetic clusters in both coral species (Buitrago-López et al. 2023).

The high degree of host genotype-specificity in microbial responses to environmental stress has enabled correlations and predictions of coral physiological states. For instance, members of the bacterial families Midichloriaceae and Spirochaetaceae varied among *Acropora cervicornis* genotypes and strongly predicted coral survival when exposed to high concentrations of nutrients and heat stress (Palacio-Castro et al. 2022). However, reciprocal transplant experiments have also shown that a notable fraction of microbiome responses can be attributed to variations in the coral host rather than the transplantation habitat itself (Chan et al. 2024; Ziegler et al. 2017, 2019). Besides such between-genotypes within-species differences, microbiomes of different host species may have a different intrinsic propensity to change following environmental change. The extent to which a coral microbiome changes with the environment may be host species-specific, a concept termed 'microbiome flexibility' (Voolstra and Ziegler 2020; Ziegler et al. 2019). In consequence, different host species have more or less flexible microbiomes. Furthermore, the environment itself may exert varying levels of selective pressures, in which more stressful conditions such as extreme heat, sedimentation, and eutrophication likely drive more substantial changes in the microbiome. Thus, not all environments induce microbiome changes and particularly benign environments may completely alleviate the dependence of hosts on their microbial communities. This is exemplified by aquaria-reared corals, which typically exhibit simplified microbiomes that bear little resemblance to their conspecific counterparts in the wild (Röthig et al. 2017; Barreto et al. 2021).

8.5 Significance for Coral Conservation

Understanding patterns of coral microbiome diversity in different environments and across host genotypes is instrumental in identifying microbiome compositions that promote coral resilience to stressors. These beneficial microbes could be used to inform the design of microbial-guided conservation and restoration efforts, such as the use of microbial therapies (e.g., probiotics) (Garcias-Bonet et al. 2024; Peixoto et al. 2022). Understanding coral microbiome assembly can also provide a baseline to detect anomalies in microbiome features that indicate stress or disturbance, enabling early intervention and targeted conservation measures. Nonetheless, the ultimate goal should be to identify beneficial microbial traits that confer advantageous holobiont phenotypes (disease resistance, stress tolerance, etc.) as microbiomes can exhibit compositional differences while still retaining certain functional traits. This requires studying the coral microbiome beyond diversity to reflect its functional contribution to the coral host. Approaches such as shotgun metagenomics, metatranscriptomics, and metabolomics combined with physiological assessments are urgently needed to fully understand the array of functions of the coral microbiome (Cárdenas et al. 2018; Rädecker et al. 2021a, b; Voolstra et al. 2024). Ultimately, leveraging the complexity of coral microbiome assemblages can inform coral restoration efforts, improving the chances of successful establishment and long-term survival.

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Part IV

Ecosystem-Scale Microbiology

9 Microbial Processes and Nutrient Uptake in the Coral Holobiont and Reef Ecosystems

Julia Saper, Jean-Baptiste Raina, Craig Humphrey, Lone Høj, and David G. Bourne

Abstract

Energy acquisition and nutrient recycling are key to the ecology, population dynamics, and stress resilience of every organism. These processes are especially important for reef-building corals as these organisms often live in nutrient-depleted water. The coral's energy budget depends on (i) the metabolites and nutrients derived from the vast diversity of microorganisms they harbour and (ii) direct heterotrophic feeding, which relies on processes occurring in the water column. Here we provide an overview of the nutrient cycling mediated by microorganisms across multiple scales in coral reefs to better characterise the multifaceted aspects of coral nutrition allowing them to thrive in nutrient deserts. We first consider how corals influence nutrient cycling in reef waters and sediments. We then focus on the microbially-mediated chemical transformations taking place in different coral micro-environments, as they are key to the recycling of nutrients and the *de novo* production of molecules essential to coral health. Finally, we describe how the direct capture of prey and particulate matter from reef waters contributes to the energy budget and stress tolerance of corals.

Keywords

Holobiont · Nutrient cycling · Heterotrophy
Microbiome · Micro-environments

9.1 Introduction

Tropical coral reefs are biodiversity hotspots that support more than 30% of all known marine species. They offer coastline protection from erosion and storms, and sustain fisheries, tourism, and recreation industries, directly impacting the livelihood of tens of millions of people worldwide (Moberg and Folke 1999; Eddy et al. 2021). The productivity and biodiversity of these ecosystems rely upon a group of ecosystem engineers, the reef-building corals. The calcareous structures deposited by these organisms create complex underwater habitats for marine species, ranging from microorganisms to fishes. In addition, corals generate high levels of primary production in otherwise nutrient-poor waters, sustaining the productivity of the food web in these ecosystems. However, reef-building corals are impacted by a myriad of anthropogenic stressors, such as eutrophication, sedimentation, deoxygenation, overfishing, and ocean acidification, that can act synergistically (Souter et al. 2021). Yet, the most imminent global threat corals face is thermal stress, induced by rising seawater temperature and increasing intensity and frequency of marine heatwaves (Hughes et al. 2017), which has impacted reefs on a global scale through mass coral bleaching events. In the current context of rapid reef loss, understanding the factors underpinning coral resilience is more important than ever.

Optimal energy acquisition is paramount to the health and resilience of all organisms. Corals acquire their energy through two main routes: (i) autotrophy, from the translocation of photosynthates by photosynthetic symbionts from the family Symbiodiniaceae; and (ii) heterotrophy, from externally sourced organic compounds. Many coral species obtain most of their energy requirements from autotrophy, which is why

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coral bleaching threatens coral survival. Coral bleaching arises when Symbiodiniaceae cells are expelled from the host tissues in response to environmental stress, depriving the host of its primary energy source. While autotrophy has been extensively studied, other important drivers of coral resilience to environmental stressors have been comparatively neglected. This is particularly true for nutrient cycling from other microbial symbionts and heterotrophic feeding. Indeed, corals live in symbiosis with abundant and diverse communities of bacteria, archaea, and protists that collectively have an enormous metabolic potential to produce and recycle essential nutrients. In addition, nutrient capture through heterotrophy directly impacts the coral hosts, Symbiodiniaceae and other associated microorganisms.

In this chapter, we explore the contributions of the different energy acquisition routes on coral health. We first focus on how corals impact nutrient cycling processes at the ecosystem scale, concentrating more specifically on microbially-mediated processes occurring in the water column and reef sediments. We then describe the contribution of its microbial symbionts, both in terms of energy and micronutrient acquisition, in the different micro-environments they inhabit. Finally, we detail the importance of heterotrophic feeding and its links with processes occurring in the water column. We illustrate how these processes can contribute to coral stress tolerance and how anthropogenic stressors impact them.

9.2 Nutrient Cycling and Processes Mediated by Microbes in Reef Ecosystems

Coral reef ecosystems are known for their high levels of gross primary productivity, however they typically occur in nutrient-depleted waters that are not very productive. Indeed, the levels of primary production originating from planktonic microorganisms can be 60 times lower in the water column above reefs than those from the benthos (Cardini et al. 2016a). In these oligotrophic waters, phytoplankton communities are dominated by nanoplankton (2–10 μm) and picoplankton (less than 2 μm) (Furnas and Mitchell 1986), which reduce the potential for strong vertical carbon fluxes (De Martini et al. 2018). In addition, the contribution of phytoplankton to the dissolved organic carbon pool of reef waters is small (Alldredge et al. 2013; Cardini et al. 2016a). Consequently, the growth of heterotrophic bacteria is rarely correlated with planktonic primary production (Rochelle-Newall et al. 2008), instead being dependent on organic matter released by the benthos (Silveira et al. 2017).

Benthic organisms release large amounts of dissolved and particulate organic carbon into reef waters (Fig. 9.1). These fluxes originate from the direct exudation of organic compounds, as well as the secretion of mucus, which forms ropes and flocs in the water column (Bythell and Wild 2011). Coral mucus is mostly constituted of a polypeptides backbone, but also contains carbohydrates, monosaccharides and other secondary metabolites (Krupp 1985; Coffroth 1990; Krediet et al. 2013), providing an ideal substrate for heterotrophic bacteria (Rublee et al. 1980; Sharon and Rosenberg 2008). Once in the water column, some of the mucus can be broken down into small particles by physical processes (e.g., wave action) (Huettel et al. 2006). This particulate matter fuels bacterial metabolism (Simon et al. 1990), preferentially supporting the growth of specific families, such as Rhodobacteraceae, Alteromonadaceae and Vibrionaceae (McNally et al. 2017). Therefore, the release of mucus by benthic invertebrates has a tremendous impact on the bacterioplankton communities in reef waters (McNally et al. 2017) and plays an important role in the carbon cycling in this ecosystem (Silveira et al. 2017; Omand et al. 2020; Nelson et al. 2023). The remaining mucus flocs that resist physical fragmentation trap drifting organic matter and microorganisms, substantially growing in weight, as well as carbon and nitrogen content (Huettel et al. 2006). The resulting large mucus flocs ultimately sink to the benthos, generating important downward carbon fluxes with subsequent benthic remineralisation of these flocs by prokaryotes further fuelling biological activity in reef ecosystems (Huettel et al. 2006; Naumann et al. 2009).

In coral reef waters, specific bacteria and archaea convert dinitrogen (N_2) to ammonium using the nitrogenase enzyme (Capone and Carpenter 1982) via a process called nitrogen fixation. As N_2 is not a biologically available form of nitrogen for other reef organisms, pelagic nitrogen fixers (or diazotrophs) can be a substantial input of "new" nitrogen, greatly supporting primary production in the water column (Bell et al. 1999; Tilstra et al. 2018). In the Pacific Ocean, the filamentous cyanobacterium *Trichodesmium* is often the most abundant and active planktonic diazotroph (Bell et al. 1999; Garcia et al. 2007; Hewson et al. 2007; Messer et al. 2017). Other planktonic nitrogen fixers include unicellular cyanobacteria and heterotrophic Proteobacteria (Hewson et al. 2007; Biegala and Raimbault 2008; Messer et al. 2017). These diverse communities can supply an important fraction of biologically available nitrogen to the overall coral reef budget, contributing to the productivity of these ecosystems (O'Neil and Capone 2008; Benavides et al. 2017). In addition, the organic nitrogen compound glycine betaine, which is an ubiquitous osmolyte across the tree of life, may account for more than 15% of the nitrogen biomass of corals (Ngugi et al. 2020). This organic compound can be synthesized by the coral holobiont, but is also taken up from the water column, making coral reefs potential sinks of this important nitrogen compound (Ngugi et al. 2020).

Pelagic prokaryotes also play important roles in sulfur cycling in coral reef waters. Indeed, high concentrations of

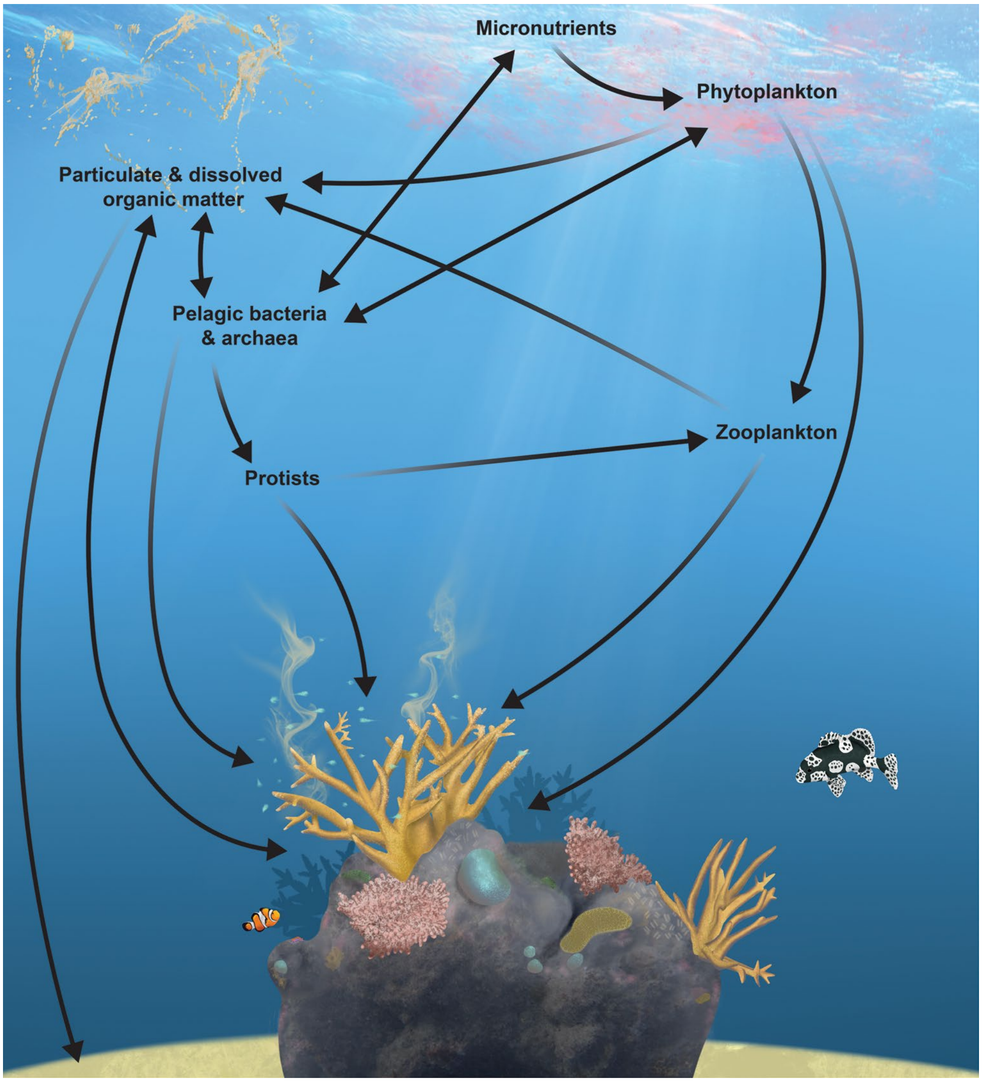

Fig. 9.1 Trophic interactions in coral reef ecosystems. Illustration: Paige Strudwick

methylated sulfur compounds, such as dimethylsulfoniopropionate (DMSP), dimethyl sulfide (DMS), and dimethyl sulfoxide (DMSO), are released by benthic organisms into the water column (Broadbent et al. 2002; Broadbent and Jones 2004). In addition, particulate matter, such as mucus rope, harbours some of the highest concentrations of DMSP ever reported in the environment (Broadbent and Jones 2004; Gardner et al. 2022). These high concentrations are relevant because marine bacteria are especially adept at exploiting chemical hotspots of DMSP (Gao et al. 2020). DMSP is a key metabolite within microbial food webs: it provides an important fraction of the sulfur and carbon demand of bacterioplankton communities (Kiene et al. 2000), it can be taken up by phytoplankton unable to produce DMSP to act as antioxidants (Theseira et al. 2020), and it mediates interspecies interactions (Seymour et al. 2010). In waters of the Great Barrier Reef, DMSP is assimilated by both bacterioplankton and phytoplankton, and likely impacts the abundance and community composition of both groups (Fernandez et al. 2021).

While most of the microbial processes occurring in the water column overlaying healthy coral reefs are mediated by the benthos, environmental perturbations and climate change can shift the structure (Glasl et al. 2019) and abundance (Nelson et al. 2013) of the pelagic bacterioplankton, which can negatively impact reef health. A well-studied perturbation is eutrophication, characterised by increased concentrations of dissolved nutrients. Elevated nutrient concentrations can induce an increase in phytoplankton biomass, which can reduce light penetration, release toxins, and reduce oxygen levels following phytoplankton bloom collapse (D’Angelo and Wiedenmann 2014). In addition, the combined effects of eutrophication and overfishing can cause a shift in the trophic structure of the reef ecosystem towards higher microbial biomass and energy use, a process called microbialization (Haas et al. 2016). These anthropogenic stressors favour the growth of benthic macroalgae over corals (Zaneveld et al. 2016), leading to larger fluxes of dissolved organic carbon into the water column (Manikandan et al. 2021). The greater availability of dissolved organic molecules fuels the metabolism of pelagic bacteria, directly threatening coral assemblages as it leads to deoxygenation events, greater CO_2 release from microbial respiration, and an increase in opportunistic pathogens (Haas et al. 2011; Nelson et al. 2013; Casey et al. 2014; Renzi et al. 2022).

Important microbial processes also take place in coral reef sediments. The substrate surrounding corals consists of fine and coarse sands, decaying coral rubbles and other reef detritus (Heil et al. 2004; Werner et al. 2006; Brocke et al. 2015). Coral sediments are exposed to high levels of particulate matter deposition (e.g., sinking mucus flocs, fish faeces) (Silveira et al. 2017), but also promote high levels of primary production (from benthic diatoms, green microalgae and cyanobacteria) in their upper surface (Werner et al. 2008). The deposition of particulates and benthic photosynthesis are two important inputs of organic matter that sustain a very high abundance of prokaryotic cells (up to 10^{10} cells mL^{-1} reef sediment), some of the highest recorded in any marine sediments (Sorokin 1973). Organic matter is efficiently remineralised in surface sediments (Alongi et al. 2008) by diverse and highly structured prokaryotic communities (Hewson and Fuhrman 2006; Rusch et al. 2009; Rusch and Gaidos 2013; Dong et al. 2022). The activity of these prokaryotic communities, structured by vertical transitions in redox states and chemical gradients, regulates benthic biogeochemistry and supports the overall productivity of coral reefs (Garren and Azam 2012).

9.3 Nutrient Cycling and Processes Mediated by Microbes Within the Coral Holobiont

Corals harbour dynamic and diverse assemblages of microbial partners, including Bacteria, Archaea, protists, Fungi and viruses (Bourne et al. 2016; Pogoreutz et al. 2020; Voolstra et al. 2024), collectively called the holobiont. Corals' microbial partners are not homogeneously distributed in their hosts but are instead partitioned between different microhabitats or compartments (Hughes et al. 2022). For example, Symbiodiniaceae are located in the gastroderm and the filamentous algae *Ostreobium* are found in the calcium carbonate skeleton. The location of these microorganisms is therefore critical to understand their functions, because it impacts their interactions with other holobiont members and ultimately their roles in supporting coral health (van Oppen and Raina 2023). The most commonly recognised compartments include the surface mucus layer (SML), tissues, gastrovascular cavity and skeleton (Fig. 9.2) (Sweet et al. 2011; Bourne et al. 2016; Hernandez-Agreda et al. 2017).

9.3.1 Nutrient Cycling and Processes in the Surface Mucus Layer

The SML harbours between 10^6 and 10^8 microbial cells per millilitre (Garren and Azam 2012). These microorganisms benefit from a rich mixture of mucosal components such as proteins, triglycerides, waxes, DMSP, and other organic compounds (Sharon and Rosenberg 2008; Bythell and Wild 2011). The composition of bacterial taxa commonly associated with the SML depends on environmental conditions and host taxonomy, but typically includes members of the Rhodobacteraceae, Oxalobacteraceae, Flavobacteriaceae, and Endozoicomonadaceae (Glasl et al. 2016; Marchioro et al. 2020). In addition to the ecological roles coral mucus plays in the water column and reef sediments, SML-associated microorganisms are a coral's first line of defence against environmental insults (Ritchie 2006; Ravindran et al. 2013). Indeed, specific SML-associated bacteria belonging to the Firmicutes and Proteobacteria (especially Gammaproteobacteria) can absorb ultraviolet (UV) radiations, and when incubated at temperatures causing thermal stress in corals, these bacteria can extend their UV absorption in the UV-A range (315–400 nm) (Ravindran et al. 2013). The overlooked UV-absorbing capacity of mucus-associated bacteria may play an important role in protecting the host against extreme UV light in summer.

Pathogenic members of the *Vibrio* genus can readily grow on detached coral mucus (Kvennefors et al. 2012), and more specifically *V. coralliilyticus* is attracted by coral mucus (Garren et al. 2014) and upregulates genes involved in virulence and biofilm formation within minutes of exposure to coral mucus (Gao et al. 2021). Given their behaviour and metabolic capabilities, these pathogenic bacteria could be expected to easily colonise the mucus of healthy corals, however they are typically rare or absent in the mucus communities derived from healthy coral colonies (Kvennefors et al. 2012). Instead, mounting evidence suggests that SML bacterial communities in healthy corals are tightly regulated by a combination of host-derived (van de Water et al. 2018) and inter-microbial interactions (Krediet et al. 2013) that contribute to their stability. Indeed, numerous bacterial genera from SML (mostly Alpha- and Gammaproteobacteria) can inhibit the growth of coral pathogens through the production of antimicrobial compounds (Nissimov et al. 2009; Shnit-Orland and Kushmaro 2009; Rypien et al. 2010). One of these compounds, tropodithietic acid, is produced by many Alphaproteobacteria and prevents the growth of *Vibrio coralliilyticus* and *Vibrio owensii* (Raina et al. 2016). The pro-

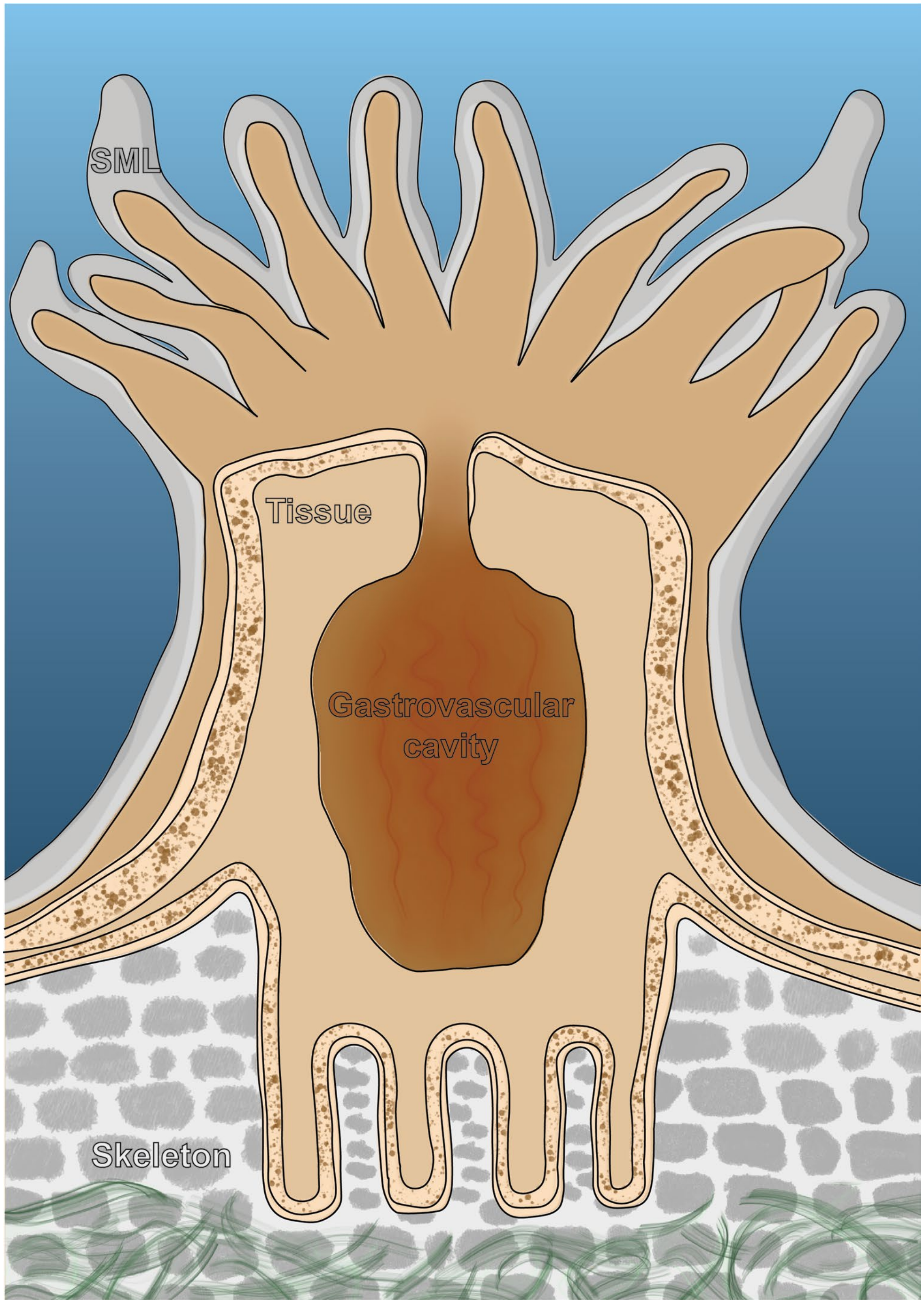

Fig. 9.2 Coral compartments harbouring microorganisms. Each compartment (i.e., surface mucus layer (SML), tissue, gastrovascular cavity, skeleton) is discussed separately in the sections below. Illustration: Paige Strudwick

duction and regulation of these antimicrobial compounds are likely key to the protection of the hosts from opportunistic or pathogenic bacteria and, by extension, likely support the health and resilience of corals.

Besides the metabolism of organic constituents of the mucus, the SML communities are also involved in the cycling of sulfur and phosphorus compounds. Indeed, levels of dissolved inorganic phosphate in coral mucus can be several orders of magnitude higher than in the surrounding seawater, and mucus-associated bacteria can consume most of these phosphate compounds in a few hours (Nakajima et al. 2015). In addition, the SML is enriched in DMSP and DMS (Broadbent and Jones 2004), hosting the largest concentrations ever measured in the environments (Broadbent and Jones 2004; Gardner et al. 2022). Coral mucus contains Alpha- and Gammaproteobacteria capable of degrading these sulfur molecules (Raina et al. 2009). DMSP concentrations in mucus increase during thermal stress (Gardner et al. 2022), which concomitantly augments the abundance of bacterial genes involved in the catabolism of this compound (Frade et al. 2016; Gardner et al. 2022). Under thermal stress, bacterial genes involved in the production of DMS (i.e., *ddd*+) become more abundant than those involved in the production of methanethiol (i.e., *dmdA*), suggesting that a larger portion of the DMSP pool is catabolized by mucus-associated bacteria to produce DMS. This is important because DMS is a climate-active gas that can enhance cloud nucleation and therefore affect solar irradiance at local scales (Ayers and Gras 1991).

9.3.2 Nutrient Cycling and Processes in Coral Tissues

The gastroderm layer of coral tissue hosts unicellular dinoflagellates from the family Symbiodiniaceae and these cells are the photosynthetic engines that support the productivity of coral reefs (Roth 2014). Indeed, while the coral host provides metabolic wastes and inorganic nutrients to Symbiodiniaceae, these dinoflagellates translocate excess photosynthates to their hosts. The amount of translocated carbon is estimated to be over 100% of the coral host's daily needs under optimal light levels (Muscatine and Porter 1977; Muscatine et al. 1984) and for many coral species constitutes its main energy source (Tremblay et al. 2012). Under these optimal conditions, both the coral hosts and their Symbiodiniaceae are nitrogen-limited (Muscatine and Kaplan 1994; Rädecker et al. 2018) and compete for the assimilation of inorganic nitrogen (Rädecker et al. 2021). Recent evidence revealed that the provision of glucose by Symbiodiniaceae simultaneously induces the up-regulation and re-localisation of glucose and ammonium transporters (Cui et al. 2023), affecting the assimilation of inorganic nitrogen required for amino acid synthesis (Cui et al. 2019). However, nutrient cycling between Symbiodiniaceae and their host is drastically impacted by thermal stress (Rädecker et al. 2021). Indeed, coral metabolism increases with temperature, and the associated increase in energy demand is compensated by the catabolism of amino acids, inducing a sudden release of ammonium that promotes the growth of Symbiodiniaceae and reduce their translocation of photosynthetically-fixed carbon (Rädecker et al. 2021). This altered nutrient cycling is a key contributor to the stress response that ultimately leads to the breakdown of the coral-algal symbiosis under heat stress (Rädecker et al. 2021).

In addition to the Symbiodiniaceae, specific bacterial genera, such as *Endozoicomonas*, *Kistimonas*, *Aquarickettsia*, and *Simkania*, form coral-associated microbial aggregates (CAMAs) in the epidermis and gastrodermis (Bayer et al. 2013; Wada et al. 2019, 2022; Maire et al. 2023). These CAMAs are postulated to be involved in complex nutrient exchanges with the host and Symbiodiniaceae, directly supporting the holobiont health and homeostasis (Wada et al. 2019, 2022; Maire et al. 2023). One of the most abundant coral-associated bacterial genera is *Endozoicomonas*, which is dominant in the microbiome of *Stylophora*, *Pocillopora*, or *Acropora* and form CAMAs within tissues (Neave et al. 2017; Wada et al. 2022; Hochart et al. 2023). *Endozoicomonas* potentially provide the coral host with different B-vitamins (Ding et al. 2016; Pogoreutz et al. 2022) and some strains are capable of degrading DMSP (Raina et al. 2009; Tandon et al. 2020; Pogoreutz et al. 2022). The abundance of *Endozoicomonas* cells within coral tissues is often tightly correlated with coral health (Bourne et al. 2008; Roder et al. 2015; Ziegler et al. 2017; Pogoreutz et al. 2018), as their abundance typically decreases in corals subjected to environmental stressors. However, the role of *Endozoicomonas* in the coral holobiont is still uncertain (Pogoreutz and Ziegler 2024), and some studies have reported that different *Endozoicomonas* strains may respond differently to environmental perturbations (Haydon et al. 2021; Tandon et al. 2022). Fortunately, a clearer picture of the compounds exchanged between *Endozoicomonas* and other holobiont members is starting to emerge (Ochsenkühn et al. 2023), which will undoubtedly allow us to accurately characterise the function(s) played by this abundant bacterial genus.

High-resolution elemental imaging has revealed that some of the bacterial aggregates present in coral tissue may contain nitrogen-fixers, which actively supply bioavailable nitrogen to Symbiodiniaceae and to the host tissues (Rädecker et al. 2022). These diazotrophic bacteria can be autotrophs (e.g., Phylum Cyanobacteria) or heterotrophs (e.g., Order Rhizobiales) (Lesser et al. 2004; Lema et al. 2012), and are taken up by coral larvae (Ceh et al. 2013; Lema et al. 2016; Benavides et al. 2017). Given that nitrogen is an important currency underpinning the stability of the coral-algal symbiosis, nitrogen fixation by diazotrophs may have beneficial or detrimental effects on coral holobiont functioning depending on the environmental conditions (Rädecker et al. 2015). Indeed, under oligotrophic conditions, nitrogen fixation can contribute up to 11% of the Symbiodiniaceae nitrogen requirements (Cardini et al. 2015), but this process increases under heat stress (Cardini et al. 2016b; Rädecker et al. 2022) or elevated sugar concentrations (Pogoreutz et al. 2017), which can further destabilise the coral-algal symbiosis. Nitrogen fixation is not the only process of the nitrogen cycle performed by coral-associated prokaryotes, but other key steps such as nitrification, denitrification, and ANAMMOX have received far less attention to date. Nitrification and denitrification rates have been measured in a few coral species (Wafar et al. 1990; Tilstra et al. 2019) and key marker genes (e.g., *amoA*, *nirS*, *nirK*) have been characterised (Tilstra et al. 2019, 2021; Xiang et al. 2022), but the exact location of these bacteria and archaea remains to be elucidated. The abundance of denitrifiers is positively impacted by the presence of Symbiodiniaceae in Aiptasia (Xiang et al. 2022), and these denitrifying communities may offset some of the nitrogen input from diazotrophs (Tilstra et al. 2019).

Although concentrations of methylated-sulfur compounds, such as DMSP and DMS, are not as high as in the mucus, their levels in coral tissues are still several orders of magnitude higher than in the surrounding seawater (Broadbent et al. 2002; Raina et al. 2013). Although most DMSP in corals is produced by eukaryotes, Alphaproteobacteria isolated from coral tissues can also produce this molecule (Kuek et al. 2022), while a wide taxonomic range of Proteobacteria can degrade this molecule to form DMS or methanethiol (Raina et al. 2009; Frade et al. 2016; Raina et al. 2017). Given the availability of DMSP as a carbon and sulfur source for coral-associated bacteria, together with the large proportion of bacteria able to metabolise this molecule, it has been hypothesised that this compound plays a predominant role in structuring the bacterial communities in reef-building corals (Raina et al. 2010).

Recently, the term Beneficial Microorganisms for Corals (BMC) was coined to identify specific microbial symbionts positively impacting the health of the coral host (Peixoto et al. 2017). Indeed, manipulative experiments have revealed that specific BMC consortia can reduce the impact of coral bleaching (Rosado et al. 2019), and increase coral survival rates after heat stress by affecting the transcriptional response of the host, influencing the microbiome structure and stabilising the photosynthetic performance of Symbiodiniaceae (Santoro et al. 2021). In addition, two specific BMC bacteria, *Halomonas sp*. and *Cobetia sp*., were recently localised in coral tissues, and their enrichment in corals correlated with improved primary productivity and photosynthetic performance of the holobiont compared to the controls (Cardoso et al. 2024).

9.3.3 Nutrient Cycling and Processes in the Gastrovascular Cavity

The gastrovascular cavity of reef-building corals is a semi-closed environment with a relatively simple structure that performs complex functions central to ingestion, digestion and reproduction processes (Hughes et al. 2022). Strong vertical gradients of oxygen, pH and nutrients are present in the cavity. During the day, photosynthesis elevates the oxygen (up to 400% air saturation) and pH (up to 9.7) levels in the upper part of the cavity (Agostini et al. 2012; Bollati et al. 2024). At night, cellular respiration makes the cavity increasingly anoxic and acidic in its lower region (Agostini et al. 2012; Bollati et al. 2024). Corals with deep polyps consistently display low pH and O_2 levels, with some anoxic zones, in the lowermost parts of their gastrovascular cavity (Agostini et al. 2012; Bove et al. 2020; Bollati et al. 2024). The presence of strong oxygen gradients in the cavity can allow corals to harbour prokaryotes that are anaerobes or facultative anaerobes and these microorganisms could aid in the catabolism of ingested organic matter and recycling nutrients (Agostini et al. 2012, Bollati et al. 2024).

The concentrations of specific nutrients are also markedly elevated in the gastrovascular cavity. Compared to the surrounding seawater, levels of phosphate can be nearly 2000 times higher, ammonium 250 times higher, nitrate 87 times higher, nitrite 37 times higher and vitamin B_{12} 30 times higher (Agostini et al. 2012). However, the source of these elevated nutrients is still unresolved, as they could originate from the ingestion of particulate matter and food, the remineralisation of particulate matter by microbial communities or the *de novo* production of vitamins by these communities. Despite the likely importance of this microhabitat in aiding in the digestion of captured prey, the specific identity and functions of the coral gastrovascular cavity microbiome are still underexplored. However, the chemical and microbial characteristics of the gastrovascular cavity of a few coral species show some similarities with the gut of higher metazoans (e.g., enrichement in putative anaerobes) (Agostini et al. 2012; Tang et al. 2020; Bollati et al. 2024).

As neither corals nor Symbiodiniaceae can produce vitamin B_{12} (Matthews et al. 2020), and enriched levels of B_{12} have been measured in the gastrovascular cavities of corals (Agostini et al. 2012), it has been hypothesised that specific microbiome members synthesise and share this essential vitamin (Peixoto et al. 2017). In support of this hypothesis, several metagenome-assembled genomes encode the complete pathway to synthesise B_{12} (Robbins et al. 2019), and most BMC also harbour the genomic potential to produce B12 (Rosado et al. 2023). In addition to the production of essential vitamins, the microbiome of the cavity may also play a role in mitigating disease. For example, *Pseudoalteromonas* spp. delivered to *Galaxea fascicularis* gastric cavities prevented infection by *V. coralliilyticus* (Tang et al. 2020).

9.3.4 Nutrient Cycling and Processes in the Coral Skeleton

The porous calcium carbonate skeleton of reef-building corals hosts taxonomically and functionally diverse microbiomes. A dense pigmented band is often visible a few millimetres underneath the coral tissue and is often dominated by filamentous green algae from the genus *Ostreobium*

(Verbruggen et al. 2017), but can also include other green and red algae (Marcelino and Verbruggen 2016). In this compartment, light is strongly attenuated, pH and oxygen levels are subjected to large daily fluctuations, and nutrient concentrations, such as inorganic nitrogen or phosphorus, are higher than in seawater (Pernice et al. 2020). The daily fluctuation in physico-chemical parameters are more pronounced in the upper part of the skeleton, but stabilise in the deep skeleton (Ricci et al. 2023). These vertical gradients are reflected in the structure of the microbial communities which are also stratified along skeletal depth (Ricci et al. 2023).

Due to the lower quantities of coral host and Symbiodiniaceae DNA in the skeleton, two recent studies have together recovered nearly 500 high-quality metagenome-assembled genomes, allowing inference of the genomic potential of endolithic microbiomes (Cárdenas et al. 2022; Tandon et al. 2023). Coral endoliths drive key steps of the nitrogen cycle, such as nitrification, denitrification, nitrogen fixation, and nitrate reduction (Yang et al. 2019; Cárdenas et al. 2022; Tandon et al. 2023), which may explain the high concentrations of nitrate previously reported in the coral skeleton (Risk and Muller 1983). In addition to nitrogen cycles, non-phototrophic carbon fixation pathways (i.e., Wood-Ljungdahl pathway, Arnon-Buchanan cycle, and 3-hydroxypropionate bicycle) have been identified in the skeleton of *Porites lutea* and *Goniastrea edwardsi*, indicating that endolithic prokaryotes likely contribute to primary production in the anoxic areas of the skeleton (Cárdenas et al. 2022). Finally, other anaerobes such as sulfate-reducing bacteria (SRB) have been reported in the coral skeleton (Yuen et al. 2013; Cárdenas et al. 2022; Tandon et al. 2023) and may also be present in other oxygen-depleted microenvironments such as the gastrovascular cavity. Through anaerobic respiration, SRBs degrade organic compounds while reducing sulfate (Muyzer and Stams 2008). Some of these SRBs may be involved in syntrophic relationships with *Prosthecochloris* (Chen et al. 2021), a group of anaerobic green sulfur bacteria abundant in the skeleton of multiple coral species (Cai et al. 2017; Yang et al. 2019). In this interaction, SRB may obtain oxidized inorganic sulfur and provide sulfide and CO_2 to *Prosthecochloris* (Chen et al. 2021).

The high nutrient cycling potential of endolithic microorganisms may contribute to bleaching resistance in some coral species in addition to direct nutritional support to the host following a bleaching event (Cárdenas et al. 2022). As more light penetrates the coral skeleton due to the expulsion of Symbiodiniaceae, the biomass and primary production of photosynthetic endolithic microorganisms (such as *Ostreobium*) increase drastically (Fine et al. 2004). During such a "bloom", endolithic algae can physically reach the host and have been shown to transfer fixed carbon to coral tissues (Fine and Loya 2002; Sangsawang et al. 2017). It has therefore been hypothesised that endolithic nutrient provision assists coral survival and even recovery following bleaching. However, blooms of endolithic algae may also stimulate skeletal erosion (Fine and Loya 2002; Ricci et al. 2019; Pernice et al. 2020), making coral colonies more susceptible to breakage.

9.4 Coral Heterotrophic Feeding and Nutrient Distribution Within Corals

The provision of photosynthates and limiting nutrients from the diverse members of the coral holobiont is not the only way corals can acquire energy, with another key source coming from heterotrophic feeding. Corals can actively graze on microscopic plankton, including picophytoplankton, protozooplankton, and bacterioplankton (Ribes et al. 2003; Houlbrèque et al. 2004a, b; Patten et al. 2011). Depleted levels of the bacterial taxa *Prochlorococcus*, *Synechococcus*, SAR11, Rhodobacteraceae and Flavobacteria have been observed in coral-dominated habitats, as compared to offshore (Nelson et al. 2011) or sandy bottom habitats (Patten et al. 2011; McNally et al. 2017). Corals therefore seem to directly influence the picoplankton communities present in reef waters, by selectively removing and promoting the growth of specific taxa (McNally et al. 2017). While autotrophic energy is provided mostly by Symbiodiniaceae, corals do require additional heterotrophically derived nutrients, such as essential amino acids, fatty acids, vitamins and metals not obtained by autotrophic means alone. Direct feeding is one potential acquisition mode for these additional nutrients along with direct provisioning by other members of the coral microbiome.

Flexible trophic strategies have supported the evolutionary success of scleractinian corals. Primitive Scleractinia, which emerged 240 million years ago, relied on heterotrophy to meet their metabolic needs until the evolution of a nutritional partnership with Symbiodiniaceae (LaJeunesse et al. 2018). The ability of a coral to derive benefits from heterotrophy depends upon its dominant nutritional strategy (Conti-Jerpe et al. 2020). Feeding modalities within Scleractinia may be summarised by three main nutritional strategies. Predominantly autotrophic corals, such as those inhabiting shallow, light replete habitats, assimilate photosynthetically-fixed carbon to meet up to 90% of their metabolic needs (Tremblay et al. 2012). Predominantly heterotrophic corals, such as those inhabiting deeper or light-limited habitats, assimilate externally sourced organic compounds to meet up to 60% of their metabolic needs (Falkowski et al. 1984). Mixotrophic corals, such as those inhabiting highly variable environments, co-vary across the resource acquisition spectrum (Houlbrèque and Ferrier-Pagès 2009). Regardless of the species-specific nutritional strategy, all symbiotic corals are in essence mixotrophs: they obtain carbon derived from both autotrophy (i.e., photosynthates) and heterotrophy (i.e., live planktonic matter, suspended particulate matter or dissolved organic matter (DOM)) to drive key biological processes. Despite the importance of both nutritional pathways, the role of autotrophy has been studied more extensively than heterotrophy in supporting coral health (Anthony and Fabricius 2000; Furla et al. 2000).

9.4.1 The Role of Heterotrophy in Mitigating Thermal Stress

Corals with adequate energy reserves tend to possess higher tolerance to temperature changes (Hughes and Grottoli 2013; Tagliafico et al. 2018) and heterotrophy may be a key factor of coral tolerance to heat stress (Grottoli et al. 2006; Tremblay et al. 2016; Conti-Jerpe et al. 2020). In bleached corals, the amount of translocated carbon drops due to the loss of Symbiodiniaceae, but also because of an increased retention of autotrophic carbon by the remaining photosymbionts (Rädecker et al. 2021). Consequently, the coral host must rely on its carbon storage and heterotrophic feeding to survive. Feeding can promote resilience by (i) boosting photosynthesis in the remaining Symbiodiniaceae cells, (ii) providing nutrients to replenish lipid stores and (iii) stimulating recovery of symbioses following heat stress events (Hughes and Grottoli 2013; Tremblay et al. 2016; Tagliafico et al. 2017). Field data revealed an increase in heterotrophy under elevated temperatures in *Favia fragum* (Grottoli et al. 2006), *Porites compressa* and *Montipora capitata* (Hughes and Grottoli 2013), resulting in lower mortality and greater recovery, despite overall declines in biomass, energy reserves (Grottoli et al. 2006), symbiont and chlorophyll densities (Hughes and Grottoli 2013).

To empirically elucidate changes to carbon budgets in thermally stressed corals, isotope markers were used to assess the metabolic adjustments of fed and unfed *Stylophora pistillata* colonies during normal conditions (25 °C) or heat stress (31 °C for 28 days). During heat stress, fed and unfed corals maintained energy homeostasis by increasing energy expenditure to sustain tissue biomass (Tremblay et al. 2016). Higher rates of respiration indicated that corals rely upon energy-rich lipid reserves and/or exogenous food sources. To compensate, less energy was allocated to growth and biomineralization, both energy intensive processes (Tremblay et al. 2016). When the heat stress subsided, unfed corals remained bleached whereas fed corals resumed normal nutritional exchanges, suggesting that heterotrophy can promote bleaching recovery by sustaining photosymbiont growth and cell concentration (Tremblay et al. 2016). Similarly, the predominantly heterotrophic *Turbinaria*, *Favites* and *Platygyra* species took longer to bleach (~7.5-degree heating weeks) than predominantly autotrophic species (Conti-Jerpe et al. 2020). Although feeding can help with the re-establishment of photosynthate translocation after an acute heat stress episode, the benefits derived via heterotrophy are not likely to be sufficient to buffer against cumulative impacts of climate change over time (Tremblay et al. 2016). The water surrounding most coral reefs is poor in nutrients and particulates (Furnas and Mitchell 1986; Cardini et al. 2016a), which likely prevents an increasing reliance on heterotrophic feeding. Nevertheless, heterotrophic feeding ability or plasticity will likely play a role in the thermal tolerance of corals under future climate scenarios (Hoogenboom et al. 2010; Imbs 2013; Tagliafico et al. 2017).

9.4.2 Energy Budget of the Coral Holobiont

Coral health depends on the benefits derived from both autotrophic and heterotrophic nutrients as well as trade-offs in nutritional strategies. A simplified coral energy budget can be expressed as the sum of autotrophic, heterotrophic and other microbial inputs (Table 9.1). This budget depends on photosynthates (e.g., glucose) in photosynthetic symbionts (C_A), heterotrophic feeding (C_H), and carbon-fixation in other microbial symbionts (C_M), which drive cellular respiration and provide metabolic currency for the coral host (R_H). In exchange, the hosts provide shelter and carbon dioxide to drive algal photosynthesis, as well as metabolic wastes to support metabolism of phototrophic (R_A) and other microbial symbionts (R_M). Partners recycle key carbon, nitrogen, sulfur and phosphorus molecules that are required for the production of biomass (P_A, P_H, and P_M). Excess nutrients are either excreted (E), often as mucus, or, allocated towards gametogenesis (i.e., reproduction) (G).

9.4.3 Autotrophic Mode

Predominantly autotrophic corals benefit from fast growth rates, resulting in high surface area to volume ratios (S/V). Some examples include branching and tabulate corals belonging to the genus *Acropora*, which are widely considered the fastest growing taxa, dominating large expanses of reef flats (Huettel et al. 2006; Gold and Palumbi 2018). However, this fast growth comes with trade-offs, as *Acropora* tends to have lower energy reserves (e.g., lipids), and less resilience to environmental stressors (Houlbrèque and Ferrier-Pagès 2009; Ferrier-Pagès et al. 2011).

9.4.4 Heterotrophic Mode

Predominantly heterotrophic corals can access and accumulate energy from a wide-range of nutrient sources, not

Table 9.1 Simplified coral energy budget (Borneman 2001), excluding the energetic outputs from competition. Inputs (left hand side of the equation) can be larger, equal or smaller than the outputs (right hand side of the equation), influencing the stability of the symbiotic interactions. $C_A + C_H + C_M >=< (P_A + P_H + P_M) + (R_A + R_H + R_M) + E + G$

Inputs	Outputs
C_A = Autotrophic inputs; photosynthates • Up to 90% of energy budget • Inefficient energy source – ≥80% C_A lost via R_A and E • High in glucose, glycerol and lipids • Low or insufficient ratios of nitrogen, phosphorus, and essential elements – Limits assimilation into host tissue biomass **C_H** = Heterotrophic inputs; food intake • Up to 60% of energy budget • Efficient energy source Increases high energy lipid stores for P_A, P_H, and G **C_M** = Microbial inputs; limiting nutrients and key micronutrients • Unquantified portion of energy budget • Additional source of primary production through alternative carbon fixation pathways • Recycling of growth-limiting elements • Production of growth-limiting molecules • Affect the health of the host	**P_A** = Translocated carbon from photosynthetic symbionts allocated to skeletal or tissue production • Positive correlation with calcification, a light-mediated process (Furla et al. 2000) **P_H** = Heterotrophically sourced carbon allocated to skeletal or tissue production • Positive correlation with tissue synthesis (Houlbrèque et al. 2004a, b) **P_M** = Translocated carbon from other microbial symbionts, allocated to skeletal or tissue production **R_A** = Respiration by photosynthetic symbionts; metabolic maintenance • Dependent on inputs from C_H **R_H** = Respiration by coral animal; metabolic maintenance • Referred to as CTAR; contribution of total acquired carbon to animal respiration (Grottoli et al. 2006) **R_M** = Respiration by other microbial symbionts; metabolic maintenance **E** = Excretion • DOC and POC; includes mucus • Drives benthic/pelagic coupling **G** = Gonad production energy allocations • If the sum of C_A, C_H, and C_M decrease, G is typically the first to disappear

accessible through photosynthesis alone (Houlbrèque and Ferrier-Pagès 2009). Some examples include *Galaxea fascicularis*, *Platygyra* and *Favia* spp. (Houlbrèque and Ferrier-Pagès 2009; Conti-Jerpe et al. 2020; Saper et al. 2023). Although feeding studies tend to focus on macrozooplankton (~200–1000 µm), the dominant planktonic fractions on coral reefs are much smaller (i.e., pico- and nanoplankton; 0.2–100 µm) and may be more important sources of nutrition. In simulated benthic assemblages, uptake of picoplankton (0.2–2 µm) by corals contributed 92% of the total nitrogen removal (Ribes et al. 2003). Similarly, consumption of pico- and nanoflagellates contributed up to 94% of the total carbon and 85% of the nitrogen ingested by *Stylophora pistillata* and *Galaxea fascicularis* colonies (Houlbrèque et al. 2004a, b). Although active capture of live prey is a significant energy source, the contributions of DOM and suspended particulate matter to coral health are also important. DOM sources include dissolved free amino acids (DFAA), carbohydrates and urea and can contribute up to 75% of daily nitrogen needs for *S. pistillata* (Grover et al. 2008). DOM uptake, as opposed to active feeding, is a non-selective, diffusion mediated process that is uncommon in animals. Lastly, suspended detrital sediments, biofilms, microalgae and protozoans, may be useful for corals in near-shore, nutrient rich environments, subjected to increased rates of sedimentation (Anthony 1999).

9.4.5 Positive Feedback Between Nutritional Modes

Despite species-specific variability in trophic strategies, the mixotrophic abilities of Scleractinian corals are paramount to their ecological success. In addition to the assimilation of host-produced CO_2, photo-symbionts benefit from nutrients derived from heterotrophic feeding, including nitrogen (Ferrier-Pagès et al. 2011; Tremblay et al. 2016). Stable isotope tracing revealed that nitrogen derived from ingested zooplankton can be transferred to photo-symbionts in under 10 min (Piniak et al. 2003). This transfer of nutrients from hosts to photo-symbionts may explain elevated chlorophyll concentrations, symbiont densities and rates of photosynthesis in fed versus unfed corals, although these results vary between species (Zhukova and Titlyanov 2003; Ferrier-Pagès et al. 2011). For example, in *Acropora*, feeding on zooplankton significantly increased both chlorophyll concentrations and symbiont densities, while in fed *Turbinaria*, chlorophyll concentrations increased independently of symbiont densities (Hoogenboom et al. 2015). Feeding seems to positively impact photo-symbiont productivity which in turn, can increase the net carbon translocated to the hosts. Heterotrophy may therefore increase photo-symbiont fitness which then enhances photosynthesis and skeletal growth via a positive feedback loop, highlighting how these two nutritional modes are inextricably linked. Finally, coral hosts can also digest excess

Symbiodiniaceae, which allows them to directly take up limiting nutrients from these cells (Wiedenmann et al. 2023).

9.4.6 Prey Capture and Digestion in Corals

Corals evolved specialized appendages used for predation and defence. Extended tentacles directly intercept planktonic food and upon detecting chemical cues, such as amino acids, or in response to contact, stinging cells called cnidocytes deploy harpoon-like organelles called nematocysts that discharge venom to stun and capture prey. The success of tentacular interception depends on environmental factors (e.g., currents, prey abundance) and morphological factors (e.g., tentacle length, nematocyst type, and density) (Fautin 2009). Because corals use the same type of nematocysts for feeding and defence, it is not possible to assess a coral's feeding ability using this trait alone (Fautin 2009). However, the location of the nematocysts can be used as a proxy for their functional importance in prey subduction and digestion. High nematocyst cell densities lining endodermal, mesenterial filaments may indicate a digestive function whereas high nematocyst cell densities lining outer epithelial surfaces of tentacles may indicate a defensive role (Fautin 2009).

The coral host can also regulate surface mucosal secretions, which trap and accumulate particulate matter. Nutrients adhering to mucus may either be directed via ciliary action to the polyp's mouth or released to the surrounding seawater, supporting benthic-pelagic coupling (Naumann et al. 2009) (Fig. 9.1). Corals that lack tentacles, such as *Mycetophyllia reesi*, rely on ciliary action to transport mucus-entrapped food particles to the gastrovascular cavity for digestion (Goldberg 2002). The release of mucus is also used by some coral species to increase their access to nutrients (Wild et al. 2004; Huettel et al. 2006). Indeed, mucus flocs that have trapped microorganisms and detritus (Huettel et al. 2006) can be recycled by corals through active feeding.

Polyp size is arguably the best predictor of a coral's trophic strategy (Falkowski et al. 1984; Conti-Jerpe et al. 2020). It is intuitive that corals with smaller polyps (e.g., <1 mm diameter in *Acropora* or *Porites*) would not be as well-adapted to the capture and consumption of prey as species with larger polyps (e.g. <300 mm in the solitary mushroom coral *Fungia scruposa*). This hypothesis was first posed by Porter who used the relationship between surface area to volume (S/V) ratio and polyp diameters to predict the "autotrophic and heterotrophic resource axes", arguing that greater S/V ratios in branching and tabulate corals optimise light interception, reducing the need for prey capture (Porter 1976). The connection between polyp diameter and heterotrophy is corroborated by stable isotope experiments with ^{15}N (Alamaru et al. 2009; Ezzat et al. 2017; Conti-Jerpe et al. 2020). Indeed, tissues sampled from the large polyp corals *Favia fragum* and *Galaxea fascicularis* had higher levels of food-derived ^{15}N than those sampled from the smaller polyp corals *S. pistillata* (Alamaru et al. 2009; Hoogenboom et al. 2015). Similarly, smaller polyp corals (e.g., *Acropora* and *Goniopora*) exhibit more overlap in host and algal ^{15}N-enrichments whereas larger polyp corals (e.g., *Platygyra*, *Turbinaria* and *Favia*) had less overlap in ^{15}N-enrichments, indicating a stronger reliance on heterotrophy (Conti-Jerpe et al. 2020). Therefore, polyp size is generally, though not uniformly, a good proxy for trophic strategy and feeding ability.

9.4.7 Internal Anatomy and Digestion of Prey

Corals break down ingested food enzymatically and mechanically in their gastrovascular cavity (Raz-Bahat et al. 2017; Hughes et al. 2022). Histology and histochemistry of *Stylophora pistillata* polyps suggest that their digestive apparatus may be more specialised and complex than previously thought (Raz-Bahat et al. 2017). Three digestive routes may be present in some species: (i) extracellular digestion via enzyme secretions (e.g., chymotrypsin) (ii) intercellular digestion through lysosomes and (iii) intercellular digestion on cell wall membranes (Raz-Bahat et al. 2017). Mesentery filaments, longitudinal membranes lining coral gut cavities, are likely the most critical components of the digestive process in corals, secreting important enzymes for ingestion, digestion, and nutrient absorption (Raz-Bahat et al. 2017).

9.5 Heterotrophy and Its Contributions to Coral Health

Corals that can increase their level of heterotrophic feeding can benefit both physiologically and energetically. Physiological benefits include enhanced tissue growth (Anthony and Fabricius 2000; Conlan et al. 2018a), skeletal growth (Anthony and Fabricius 2000; Houlbrèque et al. 2004a, b), symbiont density, and chlorophyll content (Hoogenboom et al. 2015). Coral nutrition studies have focused primarily on the interaction between diet and growth, but the use of other nutritional metrics should also be considered. This section summarises the effects of feeding on coral growth, survival and nutritional energetic metrics to provide a more holistic view of how feeding supports the health of the holobiont.

9.5.1 Feeding Effects on Growth and Survival

Growth is an important physiological metric of health because it confirms that corals can not only meet the energetic costs of maintenance tasks but also assimilate useful nutrients into biomass. Laboratory feeding studies have primarily focused on *Artemia* nauplii, rotifers and microalgae, with differential effects found on different coral species (Houlbrèque et al. 2004a, b; Osinga et al. 2012; Conlan et al. 2018b) and coral life stages (Conlan et al. 2017, 2018b). For example, *S. pistillata*, *P. acuta* and *P. damicornis* colonies fed *Artemia* nauplii showed 50 to 75% faster growth (Houlbrèque et al. 2004a, b) and can grew to twice the weight of unfed corals (Osinga et al. 2012), yet *Artemia* resulted in insignificant or negative additional growth in *Acropora millepora* (Conlan et al. 2017, 2018b). The DOM and POM levels of the used water source modulates the benefit of adding additional heterotrophic feed items (Osinga et al. 2012; Conlan et al. 2017, 2018b), and genotypic variation can significantly impact on coral growth responses (Osinga et al. 2012).

Accelerated growth rates of fed corals may enable colonies to reach critical size thresholds more quickly. Size-specific mortality is well documented, as large colonies tend to exhibit higher survival rates than smaller ones. For example, large colonies of *P. damicornis* experience significantly higher chances of survival one-year post-transplantation (Raymundo and Maypa 2004; Toh et al. 2013, 2014). In addition, fed *F. fragum* (Petersen et al. 2008) and *P. damicornis* juveniles (Toh et al. 2013, 2014) demonstrated larger post-transplantation survivorship compared to unfed juveniles. At this early life stage, a rapid increase of size is important for survival through overcoming competitive interactions, and feeding may stimulate growth and facilitate tissue fusion. Yet, in the environment, the impact of excess prey densities can be more complex, as phytoplankton blooms can reduce the availability of inorganic micronutrients for benthic organisms, negatively impacting coral resilience (D'Angelo and Wiedenmann 2014).

9.5.2 Feeding Effects on Energetic Parameters

The assimilation of heterotrophic energy sources offers more complete nutrition to corals than autotrophy, including additional sources of high-energy lipids (Tremblay et al. 2011, 2016; Tagliafico et al. 2017; Radice et al. 2019). Metabolism of lipids from tissue stores may bolster resistance to thermal stress (Imbs and Yakovleva 2012). For example, fed *C. caespitosa* (Hoogenboom et al. 2010) and *Turbinaria reniformis* (Tremblay et al. 2016) can better compensate for lower rates of photosynthesis and subsequent energetic losses. Lipid enrichment through heterotrophic feeding can help corals recover from acute bleaching scenarios, but the underlying mechanism is not well described (Tagliafico et al. 2017) and it is still unclear if manipulating heterotrophic feeds with essential nutrients can also benefit corals that are less reliant on heterotrophy. Lipid analyses that determine total lipid stores, composition of lipids and fatty acid classes could determine the health effects of dietary regimes and environmental conditions on corals (Imbs and Yakovleva 2012; Conlan et al. 2017, 2018a; Brodnicke et al. 2019; Rocker et al. 2019; Yu et al. 2021).

9.6 Summary and Future Directions

Microbial processes are central to all aspects of corals' energy acquisition, from the photosynthetic symbionts in their tissue and skeleton that translocate photosynthates, to bacteria and archaea recycling growth-limiting elements and potentially fixing carbon, to DOM uptake and heterotrophic feeding on planktonic organisms. Despite their tremendous importance for coral health and resilience, we still have an imperfect view of many facets of coral-microbe interactions. Considering benthic-pelagic coupling, it is still unclear how corals deplete or enrich specific microorganisms in the water column, and how microbial assemblages in reef environments impact the communities associated with corals. Regarding nutrient cycling in the holobiont, the functional roles and locations of important microorganisms are just starting to emerge, but most of this information has only been inferred from DNA-based sequencing so far. Finally, clearly linking the ripple effects that heterotrophic feeding has on the structure, functions and nutrient cycling mediated by the coral microbiome is still in its infancy. Although technical bottlenecks have long prevented the characterisation of nutrient exchanges between specific microorganisms and their hosts in complex symbioses, manipulative experiments and a wide range of analytical techniques are now applicable to corals. These new approaches will undoubtedly contribute to clarifying the roles and quantify the contributions of overlooked microbial partners in coral reef health.

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10

Ecosystem Microbiology of Coral Reefs

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Abstract

Coral reefs are one of the most biodiverse and productive ecosystems on Earth, as evidenced by the multitude of colorful and vibrant hard and soft corals, sponges, fish, and other reef dwellers. Beyond this visually accessible biodiversity, coral reefs host a vast array of microbial communities that play crucial roles in maintaining reef health and stability. This chapter explores the immense diversity of microorganisms including bacteria, fungi, viruses, and microeukaryotes that inhabit coral reefs. It also examines how microbial communities are structured by abiotic and biotic factors, and how microbial processes contribute to ecosystem function and resilience. This chapter provides an overview of the microbial diversity within coral reefs with a focus on non-host-associated microorganisms, the factors influencing their diversity, and the processes mediated by these microbes. Furthermore, this chapter highlights the significance of microbes as indicators for evaluating reef health and supporting biodiversity monitoring. Finally, the chapter discusses the potential of microbes as agents for active interventions relevant for conservation and biodiversity monitoring.

Keywords

Coral reef microbiomes · Coral reef microbial diversity · Reef health indicators · Reef health biomarkers · Abiotic factors · Biotic factors · Ecosystem function · Free-living microorganisms · Non-symbiotic microorganisms · Microbial processes

10.1 Introduction

This chapter delves into the fascinating field of coral reef ecosystem microbiology, focusing on the identification and exploration of microbial indicators that offer insights into reef ecosystem processes, coral health, and resilience. By examining microbial diversity and dynamics, we uncover the crucial roles microorganisms play in maintaining these delicate ecosystems (e.g., see Chaps. 2–6).

Identifying microbial indicators is vital for unraveling the complex interactions and feedback loops that sustain coral reef health. Microbes drive essential nutrient cycles, recycle organic matter, and influence nutrient availability for corals and other reef organisms (Wegley Kelly et al. 2018; Cui et al. 2023). They also contribute to the carbon budget of coral reefs, potentially mitigating the impacts of climate change through carbon sequestration processes (Lovelock and Duarte 2019) and play a role in the global nitrogen budget of the world's oceans (Ngugi et al. 2020). Additionally, microbial communities associated with corals underlie coral health and disease dynamics, influencing not only the well-being of individual corals but also impacting the overall health and stability of coral reef ecosystems (Voolstra et al. 2024). Abiotic factors (e.g., temperature, salinity, light) affect microbial community structure, which in turn impacts biotic interactions that influence the surrounding environment. Thus, microbial diversity and function are impacted by biotic and abiotic interactions that shape ecosystem function and productivity. With the growing recognition of microbes as indicators of reef health and resilience, new techniques and technologies are emerging to study and manage these microbial communities (Voolstra et al. 2024). For instance, the continuous decrease in sequencing costs now enables 'molecular monitoring' of coral

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reef processes (Hallock et al. 2004; Knowlton and Leray 2015; Pearman et al. 2019; Baer et al. 2023). Identifying molecular microbial indicators that correlate with healthy reef ecosystems is a crucial first step in developing effective methods for monitoring and managing coral reefs, facilitating targeted conservation efforts, and aiding in the restoration of degraded reef systems (Voolstra et al. 2025; Voolstra et al. 2021) (see Chap. 13 and Chap. 16).

This chapter aims to provide a comprehensive overview of the field of coral reef ecosystem microbiology. Through a synthesis of current knowledge and recent research, we will explore the diversity and functions of coral reef microbial communities, their interactions with the surrounding environment, and their potential as indicators for monitoring and assessing reef health (Fig. 10.1). Our emphasis is on environmental microbes, given that various chapters are dedicated to structure and function of microbiomes associated with the coral host. Unraveling the mysteries of the microbial world of coral reefs will undoubtedly contribute to the appreciation, understanding, and conservation of these vital ecosystems for future generations (Galand et al. 2023; Hochart et al. 2023).

10.2 Microbial Diversity in Coral Reefs

The microbial diversity associated with coral reefs is vast and complex, encompassing a wide array of microorganisms such as bacteria, archaea, fungi, microeukaryotes, and viruses (Galand et al. 2023; Rohwer et al. 2002; Thurber et al. 2017; Wegley et al. 2004). These microorganisms form intricate communities within the coral reef environment, known as the coral reef microbiome (Galand et al. 2023). Corals and other reef animals (e.g., fishes, sponges, bivalves, etc.) are hotspots of marine microbial diversity (Chiarello et al. 2020; Galand et al. 2023), making host-associated microbiomes a key focus of research (Voolstra et al. 2024; Legrand et al. 2020; Orlić 2019; Pita et al. 2018). However, to fully understand the coral reef microbiome, it is essential to include free-living microbes found in the water column and sediment, as these provide important ecosystem functions, which are detailed further in this chapter. Recognizing patterns of reef microbial diversity and interactions may help us understand and predict responses to disturbances, estimate ecosystem health, and understand nutrient dynamics. Further, a detailed inventory of coral reef microbial diversity is essential to biodiversity conservation and monitoring, as we can only preserve and protect what we know. In the following sections we discuss common reef microbes, with a focus on prokaryotes (i.e., bacteria and archaea), microbial eukaryotes (single-celled eukaryotes), and viruses.

Prokaryotes. Seawater has a concentration of 10^4 to 10^7 prokaryotic cells per mL (Whitman et al. 1998). Besides bacterioplankton, many prokaryotes associate with multicellular organisms, such as corals, sponges, etc. While some microbial groups provide beneficial functions, such as vitamin B12 provisioning (Agostini et al. 2009), others can be harmful pathogens affecting entire reefs (Ben-Haim et al. 2003; Bourne and Webster 2013; Papke et al. 2024). Estimates of prokaryotic diversity based on 16S rRNA marker gene sequencing predict between 2.72 to 5.44 million Amplicon Sequence Variants (ASVs) globally (Louca et al. 2019). However, this is probably a gross underestimation. A recent study assessed reef microbiome composition and biogeography by sampling planktonic communities, three coral morphotypes (*Millepora platyphylla, Porites lobata*, and *Pocillopora meandrina*), and two reef fish species across 99 reefs from 32 islands throughout the Pacific Ocean. This comprehensive analysis estimated that the global reef microbiome alone surpasses 2.8 million ASVs (Galand et al. 2023). This study reported the highest diversity in the coral reef bacterioplankton community in comparison to coral and fish, highlighting the tremendous diversity of free-living microbes in reef ecosystems (Galand et al. 2023). Similarly, estimates of microbial abundance in coral reef sediments far exceed those of water, with approximately 10^9 cells per mL (Schöttner et al. 2011; Wild et al. 2006). Prokaryotic diversity within reef sediments has been reported to be as diverse as terrestrial soils (Dong et al. 2023; Uthicke and McGuire 2007; Wild et al. 2006). This not only supports the overall underestimation of global prokaryotic diversity, but also highlights the massive 'dark matter' of undescribed microbes within coral reef ecosystems (Schultz et al. 2022). Coral reef prokaryotic diversity typically follows a long tail distribution, with seawater samples often containing only a few highly abundant taxa and many rare ASVs (Ma et al. 2022). Abundant taxa often show the greatest variance in relative abundance across colonies and reefs (Ma et al. 2022). This observation aligns with findings that the range of relative abundances of indicator taxa (those most effective at predicting environmental conditions and reflecting changes in reef condition), can vary widely between 0.5 and 20% in coral reef seawater (Glasl et al. 2019). Abundant prokaryotic taxa in coral reef seawater often include *Synechococcus, Prochlorococcus*, Rhodobacteraceae, and Alphaproteobacteria (Ma et al. 2022; Weber et al. 2020).

Microbial eukaryotes. Coral reefs are also home to a diverse range of symbiotic and free-living microbial eukaryotes. Notably, dinoflagellates from the family Symbiodiniaceae (see Chap. 2) form obligate symbioses with many marine organisms, most prominently reef-building corals (LaJeunesse et al. 2018). These algae are a

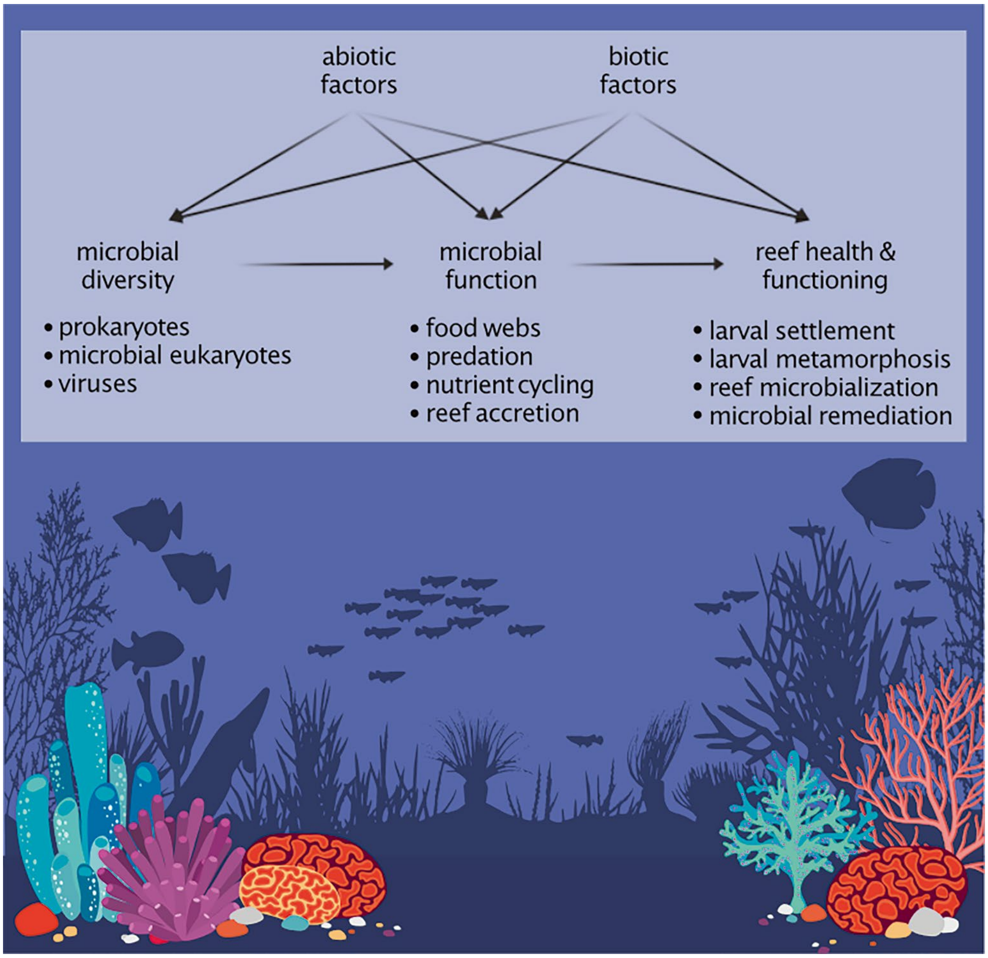

Fig. 10.1 Microbiology of coral reefs. Biotic and abiotic factors affect microbial diversity, microbial function, and reef health with microbial diversity determining microbial function that in turn contributes to reef health and functioning. Figure uses elements from macrovector/Freepik, with modifications by the authors

major focus in coral holobiont research due to their critical role in coral biology (LaJeunesse et al. 2018; Lesser et al. 2013; Muscatine et al. 1991; Weber and Medina 2012). Disruption of this symbiosis results in coral bleaching, where corals expel their algal endosymbionts, losing their primary energy source and color (Rädecker et al. 2021; Brown 1997; Douglas 2003; Scott et al. 2024; Helgoe et al. 2024). While hundreds of Symbiodiniaceae species form symbioses with corals, each coral species typically associates with only one Symbiodiniaceae species, and each coral colony generally harbors only one Symbiodiniaceae genotype (Thornhill et al. 2014; Parkinson et al. 2015; Parkinson and Baums 2014; Reich et al. 2021). Other phototrophic microeukaryotes in coral reefs include red and green microalgae, with green algae, stramenopiles, dinoflagellates, and metazoa dominating phytoplankton communities across marine habitats, including coral reefs (de Vargas et al. 2015; Eckmann et al. 2023; Wu et al. 2020). Heterotrophic microeukaryotes include fungi, ciliates, parasitic dinoflagellates, and apicomplexans (Bonacolta et al. 2023). While the phylum Apicomplexa is generally considered parasitic, some members are often found in healthy corals (Kwong et al. 2019; Moore et al. 2008). Unlike the chromerids, their photosynthetic reef relatives (Moore et al. 2008; Oborník et al. 2012), apicomplexans have only retained a non-photosynthetic plasmid (McFadden et al. 1996). Of particular interest are the Corallicolida within apicomplexans, considered potential coral endosymbionts (Kwong et al. 2019). While there are no genes for photosystems present in their plastid genome, it contains genes for chlorophyll synthesis, suggesting some interaction with light (Janouškovec et al. 2012, 2013; Kwong et al. 2019, 2021). However, as they are also found in deep-sea corals without light exposure, their exact function within the coral holobiont remains unclear (Vohsen et al. 2020). Ciliates are unicellular alveolates that include both free-living and host-associated species (Bonacolta et al.

2023; Ainsworth et al. 2017). They act as predators of bacteria, diatoms, and dinoflagellates, contributing to the high productivity in seawater (Johnson 2011; Ravindran et al. 2023). Although they are often regarded as opportunistic pathogens found predominantly in diseased corals (Ravindran et al. 2022; Bourne et al. 2008; Sweet and Bythell 2012), ciliates also perform beneficial functions, such as removing pathogenic bacteria through predation or hosting other symbiotic microbes (Ravindran et al. 2023). Fungi (see Chap. 6) are found in most coral reef microhabitats, but data on their abundance are limited, possibly due to the oligotrophic nature of the surrounding seawater (Roik et al. 2022). However, the diversity and abundance of reef-associated fungi might be much higher than current data suggest, given the multitude of microhabitats and hosts. The high diversity of Basidiomycetes and Ascomycetes in coral hosts supports this possibility (Amend et al. 2012).

Viruses. Viruses infect all organisms in coral reefs (see Chap. 5), playing an important role in regulating plankton dynamics and biogeochemical cycling (Thurber et al. 2017; Thurber and Correa 2011) (see Chap. 5). Their abundance in coral reefs is comparable to that in the open ocean, ranging from 10^5 to 10^7 viruses per mL (Payet et al. 2014; Weynberg et al. 2017; Wilhelm et al. 2010). Unlike the open ocean, where bacteriophages dominate, coral reefs host a diverse range of eukaryotic viruses as well (Cárdenas et al. 2020; Weynberg et al. 2017; Thurber et al. 2017). The water column above tropical coral reefs contains $0.5–4 \times 10^7$ per L bacteriophages and $2–7 \times 10^4$ per L eukaryotic viruses (Thurber et al. 2017). Globally, approximately 60 viral families have been found in corals (Wood-Charlson et al. 2015), and it is assumed that corals harbor a core virome of about 9 to 12 families (Thurber et al. 2017; Wood-Charlson et al. 2015). Changes in planktonic viral composition are linked to coral bleaching and disease, although the definitive underlying role in many cases is undetermined (Levin et al. 2017; Soffer et al. 2014; Sweet and Bythell 2017; Thurber et al. 2008).

10.3 Factors Influencing Coral Reef Microbial Diversity

The microbial diversity of coral reef waters is shaped by a complex interplay of both biotic and abiotic factors. Abiotic conditions such as nutrient availability, temperature, and oxygen levels create a dynamic environment that influences microbial communities. Meanwhile, biotic factors, including interactions between microorganisms and predation, further refine this diversity. Understanding how these factors collectively shape microbial communities is crucial for deciphering the intricate balance of coral reef ecosystems.

Nutrients. Nutrients are presumably among the most important drivers in shaping microbial communities in coral reef waters (Nelson et al. 2023; Haas et al. 2016; Laas et al. 2021). Coral reefs occur naturally in nutrient-poor waters, where the recycling of scarce nutrients is particularly important for sustaining microbial diversity (Vicena et al. 2022). The availability of Dissolved Organic Matter (DOM) is a central factor influencing diversity and abundance of reef microbial communities (Apprill et al. 2021; Jales et al. 2021; Laas et al. 2021). Microbes are adept at metabolizing and recycling DOM to drive essential processes such as productivity, decomposition, and biogeochemical cycling in the reef (Nelson et al. 2023). Microbe-DOM interactions considerably shape microbial community structure, influencing the diversity and abundance of benthic producers. For instance, bacterial abundance in coral reef waters typically falls between 10^5–10^6 cells per mL (Weinbauer et al. 2010; Kegler et al. 2017), but the water closest to the benthic community often shows a relative increase in abundance of bacterial taxa that are unique to this environment. This phenomenon, known as reef aura-biomes or 'coral ecosphere', refers to the unique and dynamic microenvironment formed around individual coral colonies shaped by the unique physicochemical composition of their exudates (Bourne and Webster 2013; Walsh et al. 2017; Weber et al. 2019). It extends beyond corals to encompass various benthic community members, including fleshy macroalgae and turf algae, forming distinct microenvironments with specific nutrient and oxygen conditions that favor particular microbial communities (Cárdenas et al. 2018; Walsh et al. 2017; Weber et al. 2019). For instance, unlike 'macroalgal ecospheres', coral ecospheres select for more diverse microbiomes (Apprill et al. 2021; Walsh et al. 2017), composed of more efficient bacterial community production (Nelson et al. 2013), possibly through higher nutrient content and/or more oxidized compounds (Wegley Kelly et al. 2022). Inorganic nutrients also play an important role influencing microbial metabolism. Elevated inorganic phosphate, nitrogen, and iron concentrations can shift reefs from being coral-dominated and enriched in oligotrophic alphaproteobacterial families to algal-dominated and enriched in copiotrophic microbial taxa (Haas et al. 2016; Zaneveld et al. 2016; Kelly et al. 2012). These downstream effects on the reef microbiome composition are attributed to changes in the nutrient cycle, as the selective pressure of algal exudation and potentially greater free energy leads to the dominance of larger and more numerous microbes (Zaneveld et al. 2016), a process termed microbialization (Haas et al. 2016), discussed further below and in Chap. 11.

Temperature. While nutrients are the number one driver of microbial diversity, seawater temperature has also been singled out as one of the most important drivers of coral reef microbiome composition across all entities (Bourne et al. 2016; Zhu et al. 2021). Higher seawater temperatures have been linked to changes in abundance and growth rates, as well as compositional changes in the coral reef planktonic community (Johnson and Marshall 2007; Richardson 2008). Temperature variations have been shown to mediate shifts between pathogen- and mutualist-dominated states of the coral holobiont by affecting competition between pathogens and mutualistic bacteria (Mao-Jones et al. 2010). Similarly, temperature extremes such as marine heatwaves can drive shifts in marine microbial communities, disrupting seasonal cycles, and altering niche conditions (Brown et al. 2024). Some microbes can show temperature-dependent responses to other environmental factors. For instance, for some marine fungi, seawater temperature influences their ability to tolerate salinity (Jones et al. 2022). Although the coral-associated fungal community has been shown to strongly correlate with the host (Cárdenas et al. 2022; Chavanich et al. 2022), acroporid corals host a greater diversity of fungi in warmer than in cooler water (Amend et al. 2012). This is similar to terrestrial environments, where the fungal community composition is often driven by environmental changes (Cavicchioli et al. 2019; Roik et al. 2022).

Acidification. Anthropogenic climate change not only leads to higher seawater temperatures, but also increases CO_2 absorption, leading to ocean acidification. Even small shifts in seawater chemistry can affect the reef microbiome, resulting in increased disease-associated bacteria and fungi in coral holobionts (Vega Thurber et al. 2009) as well as shifts in microbial films on crustose coralline algae, which threaten reef stability as pH decreases (Webster et al. 2013). The effects of lowered pH on free-living microbes are not yet fully understood, but calcifying phytoplankton like coccolithophores are likely to be affected (O'Brien et al. 2016).

Oxygen. Recent mass mortality events linked to low oxygen levels highlight oxygen as a crucial and sometimes limiting factor in coral reef environments (Hughes et al. 2020). At the reef scale, dissolved oxygen concentrations generally vary from 50% to over 200% of air saturation, equating to 3.4–13.6 mg O_2 per liter at 27 °C, depending on the location and time of day (Nelson and Altieri 2019). Such broad variations in oxygen levels impact the composition and activity of microbial communities across different reef microhabitats, from the water column to the sediments (Rusch et al. 2009; Spietz et al. 2015; Doyle et al. 2022). Furthermore, elevated sea surface temperatures trigger microbial growth, raising the oxygen demand of both microorganisms and larger reef organisms. This increased demand can lead to hypoxic conditions, which in turn increase the risk of disease and mortality among reef organisms (Alderdice et al. 2022; Gregg et al. 2013; Haas et al. 2013).

Seasonal, geographic, and large-scale variation. Coral reefs across different regions or depths host distinct microbial communities due to variations in environmental conditions. Even within a single reef, topologically different areas can support distinct microbial niches. For example, outer shelf reefs of the Great Barrier Reef have shown higher microbial richness in surface waters compared to mid-shelf or inshore reefs (Frade et al. 2020). Similarly, reef sand-associated microbial communities are shown to be influenced by the sediment's mineralogical composition, oxygen content, and depth (Schöttner et al. 2011). Additionally, the reef water microbiome experiences seasonal variations (Roik et al. 2016). Viruses in coastal waters, for instance, are more abundant in summer and fall than in winter (Bergh et al. 1989; Jiang and Paul 1994; Wommack and Colwell 2000). In the Sargasso Sea, viral abundance patterns are linked to water column stability and bacterial host distribution (Parsons et al. 2012), a trend that may apply to coral reef-associated viruses as well. Similarly, seasonal differences have also been noted in dominant protist taxa across various coral reefs in the South China Sea (Zhu et al. 2021).

Biotic interactions. While abiotic factors account for the largest portion of compositional variation in microbial communities of reef water, biotic interactions also play a crucial role in shaping these microbiomes. One way to understand these interactions is through the concept of the 'plankton interactome', which refers to the complex network of interactions among planktonic organisms (Lima-Mendez et al. 2015). For example, bacteria-bacteria co-occurrence patterns have been documented in water columns of artificial and natural reefs (Fang et al. 2022; Frade et al. 2020). Similarly, co-occurrence patterns between microbial eukaryotes and bacteria can be influenced by environmental changes, such as seasonal variations and upwelling processes (Wu et al. 2020; Zhu et al. 2021, 2023). Patterns of co-occurrence are also observed between bacterial and bacteriophage populations. In the 'kill-the-winner' (KtW) strategy (Suttle 2005; Thingstad 2000; Chen et al. 2021), viruses regulate host abundances through density-dependent lytic predator–prey dynamics. For instance, it is estimated that viral lysis eliminates between 24% and 367% of the bacterial biomass daily in coral reefs of Moorea (Payet et al. 2014). However, a different relationship occurs when some viruses infect their hosts without killing them, known as the 'piggyback-the-winner' (PtW) strategy (Knowles et al. 2016; Silveira et al. 2021). This approach leads to the highest prevalence of lysogenic viruses when bacterial densities are high. PtW has been documented in degraded coral reefs undergoing microbialization, typically accompanied with higher bacterial densities (Haas et al. 2016; McDole Somera et al. 2016) (discussed further below and in Chap. 11). In this regard, it has been proposed that lytic viral predation may serve as a defense mechanism against coral reef microbialization by regulating bacterial biomass (McDole et al. 2012).

10.4 Microbial Mediation of Food Webs and Nutrient Cycles in Coral Reef Ecosystems

Nutrient and energy transfers in coral reef ecosystems are intricate and span multiple trophic levels, reflecting the complex web of interactions among the diverse reef organisms. The foundation of coral reef food webs consists of four trophic levels with hundreds of feeding interrelationships that change over time and with environmental events (Glynn 2004). Primary producers are represented by cyanobacteria, phytoplankton, macroalgae, and benthic organisms that establish symbioses with photosynthetic microalgae (i.e., corals, anemones, clams). They capture energy from the sun and convert it into chemical energy that can be used by herbivores that feed on algae and corals (i.e., parrotfish, surgeonfish, sea urchins), carnivores that consume herbivores (i.e., snappers, groupers), and apex predators that eat carnivores (i.e., sharks, rays). These trophic relationships support the overall functioning of these ecosystems through four complementary ecological processes: primary production and herbivory, nutrient uptake and release, secondary production and predation, and calcium carbonate production and bioerosion (Brandl et al. 2019). Microbes are among the most dynamic players in coral reef trophic webs and their vital role in supporting these four ecological processes has been documented for decades (Hatcher 1990; Moriarty 1979; Silveira et al. 2017).

Primary production and grazing: Primary production rates in coral reefs are comparable to those of the most productive ecosystems (56 to 1696 mmol C m^{-2} d^{-1}), but most of the organic matter produced is retained and recycled within the organisms that make up the ecosystem (Alldredge et al. 2013; Ma et al. 2015; van Hoytema et al. 2016). Benthic macroalgae, turf algae, cyanobacterial mats, and Symbiodiniaceae-associated organisms, such as corals and sponges, are the key contributors to gross primary production (van Hoytema et al. 2016; Cardini et al. 2016). In contrast, pelagic primary production, mainly by phytoplankton, only contributes up to 13% of C contribution to the reef (Alldredge et al. 2013). Furthermore, benthic microalgae, cyanobacteria, and endolithic phototrophs (i.e., *Ostrobium* algae as well as green and sulfur bacteria) are notable contributors to primary production in dead coral substrates and sediments with production rates between 1.6 and 4.8 μg C μg chl^{-1} day^{-1}) (Tribollet et al. 2006; Casareto et al. 2008; Heil et al. 2004; Werner et al. 2008). Anthropogenic activities, such as overfishing and eutrophication, can prompt changes in the benthic organism composition from a coral- to an algae-dominated community (Rädecker et al. 2015; McCook 1999). These shifts can determine primary production rates, with unimpacted reefs having scleractinian corals as primary producers, while impacted reefs having macroalgae as primary producers (Rädecker et al. 2015; Owen et al. 2021). Following primary production, biomass can be consumed, decomposed, exported beyond reef boundaries, or accumulated in the form of dead organic matter (detritus). Coral reefs are characterized to have high production rates (Crossland et al. 1991) and low accumulation rates of detritus (Alongi 1988). This is in part because of the high detritivore and decomposition rates by microbes (Cebrian 2002; Mumby and Steneck 2018).

Secondary production and predation: Although corals experience relatively low grazing pressure, they release vast amounts of mucus and other DOM. Corals can exude up to half of the material translocated from algal symbionts (Hatcher 1988; Leonard Muscatine and Cernichiari 1969; Naumann et al. 2010). This N- and P-enriched DOM is primarily broken down by microbes in the pelagic microbial loop, stimulating heterotrophic microbial metabolism in the water column, which explains microbial growth rates of up to 50 times higher than those in open ocean communities (Silveira et al. 2017; Allers et al. 2008; Nakajima et al. 2009). The efficient flow of autochthonous carbon from exudates to bacterial biomass and then to higher trophic levels partially explains why healthy coral reefs have a greater biomass of consumers than producers (inverted biomass pyramid) (McCauley et al. 2018; Sandin et al. 2008; Bradley 2016; Woodson et al. 2018). Coral exudates are used more efficiently by heterotrophic bacteria than algal exudates (18% vs. 6% bacterial growth efficiency; (Nelson et al. 2013)). Microbial growth efficiencies determine the amount of carbon transferred to higher trophic levels, and small imbalances in microbial biomass can result in shifts in energy reallocation in the ecosystem. Reefs with high human impact have a larger proportion of the energy consumed by microbes, while in pristine reefs, energy is allocated in fish compartments (McDole et al. 2012). This shift in the ecosystem's trophic structure towards higher microbial biomass and energy use is known as the 'microbialization' of coral reefs in response to anthropogenic impact (Haas et al. 2016) (discussed further below and in Chap. 11).

Nutrient uptake and release: Microbes play a vital role in nutrient cycling in coral reefs. In particular by breaking down nutrients released into the water column by benthic communities, making them available to other organisms. For example, microbes play key roles in the cycling of nitrogen and sulfur in coral reefs. Nitrogen transformation from N_2 fixation, conversion of ammonium to nitrate, and removal of nitrogen from the reef ecosystem through denitrification are essential for reef health and productivity (Cardini et al. 2015; Pogoreutz et al. 2017; Rädecker et al. 2022). Microbes also drive sulfur cycling in coral reefs, transforming inorganic sulfur into organic compounds, such as DMSP (Raina et al. 2009). In healthy reefs, most dissolved organic carbon (DOC) is autochthonously exudated from both algae and corals. Exudated DOC is highly labile and rapidly consumed by ambient assemblages of reef bacterioplankton (Haas et al. 2011; Nelson et al. 2013; Nakajima et al. 2017). DOC release by the different benthic primary producers differentially influences microbial diversity and activity (Haas et al. 2011). Fleshy algae-dominated as opposed to coral-dominated reefs are associated with an increase in the abundance of opportunistic and pathogenic microbes (Cárdenas et al. 2018; Nelson et al. 2013; Silveira et al. 2017).

Calcium carbonate production and bioerosion: Microbes play a role in both the production and erosion of calcium carbonate, which is the main component of coral skeletons and other calcifying organisms (Andersson and Gledhill 2013). For instance, *ex-hospite* calcification of Symbiodiniaceae in partnership with *Neptunomonas* sp. and *Pseudoalteromonas atlantica* is a significant source of calcium carbonate production in coral reef ecosystems (Frommlet et al. 2015). *Ex-hospite* calcification is more efficient than calcification within a coral host because it is not limited by the availability of resources within host tissues and can occur in a wider range of environments than calcification within a coral host (Frommlet et al. 2015). On the other hand, bacteria colonize and penetrate carbonate substrates by releasing acids, thereby contributing to carbonate erosion (Tribollet 2008). As oceans get warmer, metabolic rates will also increase, leading to an increase in both bioerosion and microbial remineralization (Andersson and Gledhill 2013).

10.5 Importance of Microbial Diversity and Activity in Coral Early Development

Microbes in coral reef ecosystems not only participate in trophic interactions, but also support the overall functioning of coral reef ecosystems by modulating symbiotic processes. Examples include the production of compounds that influence the recruitment, larval settlement, and metamorphosis of marine organisms.

Bacteria modulate larval recruitment and settlement. Larval settlement is a crucial developmental milestone in the life cycle of numerous marine invertebrates (including sponges, cnidarians, bryozoans, mollusks, and crustaceans among others), and it is essential for the successful recruitment of new individuals into populations (Gutierrez et al. 2018; Webster et al. 2011; Dobretsov and Qian 2006; Alfaro et al. 2011; Anderson and Epifanio 2009). Larval stages of these invertebrates can sense biofilm chemical cues that guide them to settle on the most suitable surfaces via three major routes: inductive molecules, exoenzymes, and physical viral-like structures (Dobretsov and Rittschof 2020). Inductive molecules include those involved in Quorum Sensing (QS) from established biofilms and consortia (Dobretsov et al. 2007, 2009). For instance, Acylated Homoserine Lactones (AHLs), a major class of QS molecules, are involved in a diverse range of bacterial behaviors. Although their role in larval settlement is starting to become more apparent, the specific mechanisms underlying these responses are still unknown (Huang et al. 2007; Tait and Havenhand 2013; Cicirelli et al. 2014). The release of exoenzymes during microbial uptake of nutrients results in degradation products that provide nutrition for larvae and indirectly act as cues for settlement (Bonar et al. 1990). Finally, *Pseudoalteromonas luteoviolacea* produces phage tail-like structures called tailocins, that promote larval settlement in the serpulid polychaete *Hydroides elegans* (Shikuma et al. 2014). Intriguingly, while various bacteria spur *H. elegans* settlement, they utilize different mechanisms. The tailocin-dependent settlement appears to be specific for *P. luteoviolacea*, while other bacteria including *Cellulophaga lytica*, *Bacillus aquimaris*, and *Staphylococcus warneri* rely on the production of bacterial lipopolysaccharide (LPS) and extracellular vesicles (Freckelton et al. 2017, 2022). Unlike tailocin-dependent settlement, LPS and extracellular vesicles may be broadly important for marine invertebrate larval recruitment, and we have yet to establish their prevalence among bacterial taxa and hosts. Further examples include coral settlement induced by photodegradation of the bacterial pigment cycloprodigiosin (CYC) as a settlement cue for the coral *Leptastrea purpura* (Petersen et al. 2023) and the production of Tetrabromopyrrole (TBP) by *Pseudoalteromonas* species (Sneed et al. 2014) to induce settlement in several corals.

Bacteria modulate larval metamorphosis. Besides guiding settlement, marine invertebrate larvae can detect specific bacterial signals, including metabolites or direct contact, that induce metamorphosis into adult forms. Although metamorphosis is pivotal across animal taxa, the precise bacterial cues and mechanisms governing this transition remain enigmatic for most species. Intriguingly, different triggers can elicit metamorphosis within a species. For instance coral larvae respond to both the TBP produced by *Pseudoalteromonas* sp. (Alker et al. 2023) and crustose coralline algal metabolites (Gómez-Lemos et al. 2018). Likewise, independent pathways involving bacterial lysophospholipids and exopolysaccharides stimulate metamorphosis in *Hydractinia* (Guo et al. 2021). A fascinating mechanism used by *Pseudoaltermonas luteoviolacea* is directly injecting proteins into tubeworm larvae via syringe-like structures called metamorphosis-associated contractile structures (MACs) (Shikuma et al. 2014). While some bacteria like *P. luteoviolacea* use MACs, others including *Cellulophaga lytica*, *Bacillus aquimaris,* and *Staphylococcus warneri* produce inductive extracellular vesicles to trigger metamorphosis (Freckelton et al. 2017).

10.6 Microbial Effect on Reef Health and Resilience

Coral reefs are an intricate and vibrant ecosystem that exists within a balance of interactions between its multiple components. This section focuses on reef health and resilience at the ecosystem level. As outlined above, reef resilience is tightly intertwined with the microbial community, with microorganisms playing a fundamental role in nutrient cycling, carbon fixation, diseases, settlement regulation, etc. (see the relevant other chapters in this book). In the following section, we highlight how ecosystem-scale microbial processes contribute to the resilience or, conversely, deterioration of coral reefs.

Reef microbialization, characterized by a shift towards higher microbial biomass triggered by increased DOC, is one example of disruption at the reef ecosystem level resulting from microbial processes. DOC from coral and algae differs in neutral sugar content (Nelson et al. 2013), prompting heterotrophic bacteria to adjust their metabolic activities (Cárdenas et al. 2018). When consuming DOC from macroalgae, opportunistic bacteria switch to the energy-intensive Entner–Doudoroff and pentose phosphate pathways (Haas et al. 2016; Cárdenas et al. 2018), This metabolic shift lowers bacterial metabolism efficiency, leading to increased bacterial biomass and reduced energy for higher trophic levels (Haas et al. 2016). Moreover, elevated microbial biomass also raises respiration rates, CO_2 levels, and the risk of hypoxia, which can negatively impact reef health (Haas et al. 2016). In addition, viruses proliferate in response to the increased organic matter, which can reduce the abundance of beneficial microbes that protect corals from disease (Silveira et al. 2017) (see Chap. 11).

As highlighted in this example, the interaction between free-living microbes and organisms has significant functional consequences for the holobiont phenotype (Webster and Reusch 2017). Although we focused on negative interactions in the previous paragraph, there is growing consensus that microbes have the potential to be harnessed as a tool to improve reef resilience (Voolstra et al. 2021; Peixoto and Voolstra 2023). The concept of putatively beneficial microorganisms for corals (pBMCs), i.e. coral microorganisms theorized as able to enhance coral fitness through their symbiotic relationships, is well established. Furthermore probiotic therapy, i.e. the administration of live microorganisms that provide health benefits to the host when consumed in adequate amounts, has been demonstrated to improve resilience in many species, including coral (Santoro et al. 2021; Rosado et al. 2019; Peixoto et al. 2017). Manipulation or restoration of the microbiome has been shown to increase thermal resilience, support bioremediation, and enhance growth (Doering et al. 2021; Fragoso Ados Santos et al. 2015; Morgans et al. 2020; Rosado et al. 2019; Zhang et al. 2021; Voolstra and Ziegler 2020; Silva et al. 2021). The rapid adaptation to changing environments in conjunction with our ability to manipulate the genetic constitution of microbes offers various active intervention applications (Voolstra et al. 2021).

10.7 Conclusion

The coral reef microbiome represents a vast and intricate network that underpins critical ecological processes in coral reef health, function, and resilience. The delicate balance of these microbial communities is shaped by a myriad of factors (i.e., nutrient availability, temperature, pH, and biotic interactions), making them sensitive indicators of environmental change. Coral reefs are home to a remarkable diversity of prokaryotes, with recent studies revealing numbers that surpass total global estimates and hinting at a wealth of undiscovered microbes. To fully appreciate biodiversity, understand microbial functions, and develop effective conservation and restoration strategies, it is essential to examine microbial diversity across all domains.

Microbes are the engines driving the entire reef food web. Key groups like cyanobacteria, microalgae, and endolithic phototrophs contribute significantly to primary production. Their roles extend to crucial nutrient cycling processes, including nitrogen and sulfur cycling, and they facilitate secondary production via herbivory and predation. Microbial activity is also vital for carbonate production, bioaccumulation, and bioerosion. The efficient decomposition of detritus by microbes ensures minimal detrital accumulation, while their enhanced growth rates in the nutrient-rich reef environment contrast sharply with the lower rates observed in the open ocean. Moreover, microbes influence symbiotic relationships, affecting the recruitment, settlement, and metamorphosis of larvae. These intricate interactions and high microbial activity are fundamental to maintaining the dynamic balance and resilience of coral reefs.

As coral reefs face unprecedented threats from global warming, ocean acidification, and local anthropogenic stressors, understanding the role of microbial diversity becomes increasingly crucial. The concept of reef microbialization highlights how shifts in microbial communities can fundamentally alter reef ecosystem functioning, while emerging research into beneficial microorganisms for corals (pBMCs) and probiotic interventions offers potential avenues for enhancing reef resilience. Moving forward, integrating reef microbial ecology into coral reef conservation strategies will be essential. By deepening our understanding of these microscopic yet mighty players in reef ecosystems, we may unlock new approaches to protect and preserve these vital marine habitats. The future of coral reef conservation may well depend on our ability to work with and harness the power of the reef microbiome (Peixoto et al. 2022).

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11 Coral Reef Microbialization and Viralization Shape Ecosystem Health, Stability, and Resilience

Jason Baer and Forest Rohwer

Abstract

Microbes mediate the flow of organic carbon through aquatic ecosystems, and the structure of microbial communities is linked to ecosystem health and functioning. Globally increased inputs of organic matter (OM) over the past several decades have resulted in widespread degradation and trophic simplification of aquatic ecosystems, including coral reefs. As ecosystems degrade, they become increasingly dominated by microbial biomass (usually enriched with potential pathogens) and energy use, a phenomenon termed microbialization. The enhanced microbial respiration of OM that underlies microbialization results in deoxygenation, acidification, and increased outbreaks of disease that, in turn, cause mortality of macrofauna and erode benthic structural complexity. In this chapter, we review the biochemical drivers and impacts of microbialization on coral reefs and discuss how microbialization is reinforced by biological feedbacks and global climate change. We also introduce the countering process of viralization and discuss how *in situ* experimental tools may improve reef health.

Keywords

Microbialization · Deoxygenation · Coral reef Biogeochemistry · Metabolism · Microbial ecology Eutrophication

11.1 Introduction

Healthy ecosystems are organized hierarchically in trophic levels, enabling energy fixed by primary producers to be channeled between the microbes and the macrobes (Odum 1968). Ecosystems degrade when this organization is disrupted, resulting in a shift in ecosystem energy allocation from larger organisms and the macro-scale processes they support to the microbes. This shift in ecosystem trophic structure towards higher microbial activity and energy use is known as ***microbialization*** and is a prominent mediator of decline in coral reef ecosystems (Haas et al. 2016). Microbes, owing to their sheer numbers and high metabolic rates relative to their size (DeLong et al. 2010), are the primary agents of energy transfer in ecosystems and determine the biogeochemical landscape of coral reefs (Carlson et al. 2007; Moriarty 1979; reviewed in Nelson et al. 2023). When tightly regulated through trophic control, coral reef microbes recycle essential nutrients and shunt energy in the form of dissolved organic matter (DOM) up to higher trophic levels, facilitating high productivity and biodiversity in nutrient-poor waters (Odum and Odum 1955). Yet, coral reef microbialization has shifted the role of microbes from trophic links to energy sinks, diverting the flow of ecosystem energy into the microbial food web at the expense of the macrobes. Threats currently facing coral reefs, including deoxygenation, acidification, and trophic downgrading, are a consequence of this microbial expansion.

Coral reef ecosystems generate more than $400 billion in annual revenue by way of ecosystem services that provide food, coastal protection, and tourism to coastal communities (Moberg and Folke 1999; De Groot et al. 2012; Costanza et al. 2014). Coral reefs are currently in decline globally,

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with reef-building corals being replaced by alternative benthic assemblages composed of turf- and fleshy-macroalgae (Hughes 1994; Smith et al. 2016). Transitions to algal dominance facilitate coral reef microbialization via the **DDAM** positive feedback system (dissolved organic carbon (DOC), disease, algae, and microorganisms (Kuntz et al. 2005; Kline et al. 2006; Barott and Rohwer 2012). DDAM is initiated by local eutrophication and overfishing (McCook 1999; Zaneveld et al. 2016), which release controls on algal growth and enable macroalgae to dominate over corals on the reef benthos (Fig. 11.1). **Macroalgae release labile organic carbon and bubble off photosynthetic oxygen, creating a benthic environment rich in electron donors (DOC) and depleted of electron acceptors (O_2).** The increased **electron donor to acceptor ratio (e^{-}DAR)** in reef water provides an abundant carbon source for microbial consumption with relatively less oxygen; conditions that favor rapid microbial growth (Haas et al. 2011; Silveira et al. 2019). Increased e^{-}DAR selects for copiotrophic, virulent microbial communities that create suboxic zones and cause disease, contributing to coral mortality and freeing up benthic space for further algal overgrowth (Smith et al. 2006; Haas et al. 2013a; Silveira et al. 2019, 2020). The loss of corals and other sessile benthic invertebrates, which prey on microbes via suspension feeding, reduces organic matter (OM) recycling to higher trophic levels and compromises benthic-pelagic coupling processes connecting reef biogeochemical cycles (Bak et al. 1998; McNally et al. 2017).

One initiating factor for DDAM is a loss of predation pressure by fish, preventing the transfer of photosynthetically fixed carbon between the microbial and macrobial food webs. Predation pressure is a stabilizing force in coral reef ecosystems: at the macro-scale, predation by large fish controls the abundance and distribution of smaller fish (DeMartini et al. 2008; Sandin et al. 2008; Boaden and Kingsford 2015), including reef herbivores, which facilitate the transfer of algal production to higher trophic levels (Mumby et al. 2006; Zgliczynski and Sandin 2017; McCauley et al. 2018). At the micro-scale, viral predation via viral lysis controls microbial densities, preventing energy from accumulating in the microbial food web (Wilhelm and Suttle 1999; Suttle 2007). Indeed, coral cover

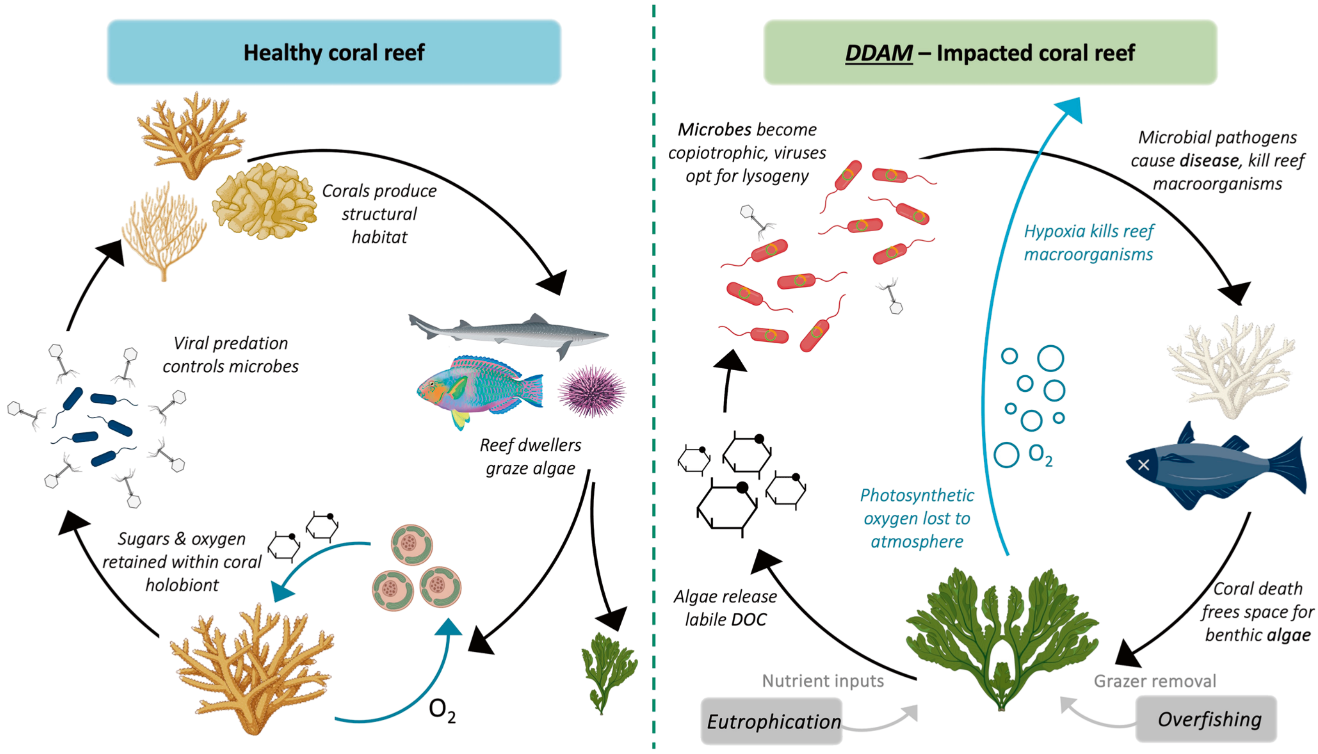

Fig. 11.1 Positive feedback loops reinforcing coral reef health (left) and degradation (right). (Left Panel) On healthy coral reefs, corals use sugars and oxygen produced by photosynthesis in endosymbiotic zooxanthellae to build three-dimensional habitat for reef macrofauna, including herbivorous invertebrates and fish. Herbivory pressure keeps the cover of turf- and fleshy-macroalgae low, facilitating coral dominance. Coral reef microbes are maintained under trophic control by lytic viruses. (Right Panel) Reefs degrade according to the DDAM positive feedback loop. Local overfishing of herbivores and eutrophication enable the overgrowth of fleshy macroalgae, which release dissolved organic carbon, stimulating the growth of heterotrophic microbes which reduce oxygen concentrations and cause disease, killing corals and freeing space for further algal overgrowth. A switch among coral reef viruses to lysogeny facilitates further microbial community expansion, shunting algal photosynthetic production into the microbial food web and preventing transfer to higher trophic levels

has been observed to be highest on reefs with high predator fish biomass and high virus-to-microbe ratios (VMRs, Box 11.1), indicating the combined effects of predation pressure by fish and viruses are instrumental in coral reef health and stability (Silveira et al. 2023). However, herbivory pressure by fish and lytic predation by viruses are reduced on overfished, algal-dominated reefs, accelerating transitions to algal and microbial dominance. Coral reefs experience **viralization**, the counter process to microbialization, when viral control of microbial growth and a robust and structured fish community retains nearly 100% of ecosystem energy in the macrobial food web. **Reef transitions from healthy, viralized states to degraded, microbialized states are thus initiated by a loss of algal grazing pressure, mediated by resulting shifts in reef biochemistry towards high e^-DAR, and accelerated by the loss of viral predatory control on microbial expansion.** These transitions to high e^-DAR and microbialization can likely also be initiated by other stressors, such as ocean warming events and hurricanes, which cause widespread mortality and divert organic carbon flows into the microbes.

Microbialization is a natural feature of ecosystems with consistent inputs of organic matter (OM) and can play an important role in OM recycling and biogeochemical cycles. However, globally increased inputs of OM to coastal environments have shifted the role of microbialization from a localized and transient phenomenon to a widespread and persistent threat to coastal ecosystems. On degraded coral reefs, the microbial food web is predicted to process and accumulate almost 100% of ecosystem energy (McDole et al. 2012; Somera et al. 2016), leading to losses in the diversity of macrobes, acute and chronic conditions of hypoxia and microbial acidification, and more recently to tropical dead zones (Altieri et al. 2017; Alteri et al. 2019). Here, we place coral reef degradation in the much wider context of global microbialization and show that seemingly disparate phenomena mediating ecosystem decline are linked to the unchecked expansion of the microbes. We show how an increase in e^-DAR, caused by algal release of labile carbon and several mechanisms of deoxygenation, reshape the biochemical reef environment to favor microbial dominance. Next, we present Coral Reef Arks, an experimental tool to reduce e^-DAR, and thus microbialization, on coral reefs and discuss potential interventions for restoring ecosystems in a microbial world.

Box 11.1 Virus-to-Microbe Ratio and Coral Reef Microbialization

The virus-to-microbe ratio (VMR) is an outcome of the interactions between microbes and their viral predators and is used as a proxy for microbialization (McDole et al. 2012; Silveira et al. 2023). Calculated as a ratio of the abundance of free viruses to microbial cells, VMR can be used to approximate the relative frequency of two dominant modes of viral infection, lysogeny and lysis, among microbial communities. While canonical Lotka-Volterra predator-prey dynamics predicted the frequency of lysogenic infections in a microbial community to decrease with increasing microbial abundance (more prey encounters = more lysis), analysis of VMRs from diverse global environments provided evidence that VMR decreases with increasing cell densities (Knowles et al. 2016). This finding led to the development of the Piggyback-the-Winner hypothesis, which predicts viral lysis as a dominant infection strategy at intermediate bacterial densities (Thingstad 2000) and predicts lysogeny to dominate at both high and low bacterial densities (Fig. 11.2, Knowles et al. 2016; reviewed in Silveira et al. 2021).

Coral reefs experiencing microbialization display reduced VMRs relative to healthy sites (Knowles et al. 2016), suggesting a decrease in viral lytic predation pressure which facilitates microbial expansion. Metagenomes from reefs with low VMRs are enriched in prophages and phage-encoded virulence genes, confirming the increase in the frequency of lysogenic infection on these reefs and highlighting lysogeny as a primary driver of coral reef microbialization and decline (Knowles et al. 2016; Touchon et al. 2016; Little et al. 2020). While VMR serves as a useful proxy for viral lytic/temperate dynamics and thus for the magnitude of viral predation pressure on microbial communities, genomic markers including the presence of integrases, excisionases, lysis repressors and known prophage sequences are still the best proxies to identify lysogens and temperate phages in ecosystems (Luo et al. 2020; Silveira et al. 2020).

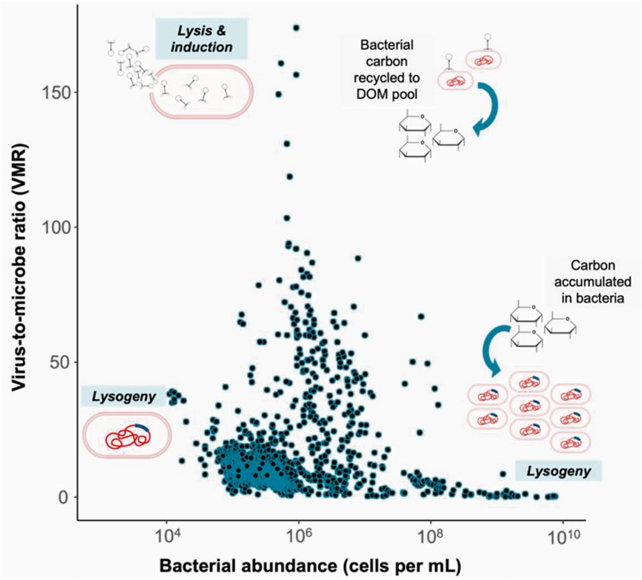

Fig. 11.2 Narwhal plot showing the relationship between virus-to-microbe ratio (VMR) and microbial abundance. At high VMRs, more abundant free viruses relative to microbial cells indicate high microbial turnover rates via viral lysis, contributing to the recycling of bacterial carbon back to the DOM pool. At low VMRs, free viruses are less abundant relative to microbial cells, indicating a switch to a latent viral infection strategy, lysogeny, which results in the accumulation of ecosystem carbon in the microbial compartment and accelerates microbial expansion. Figure modified from Silveira et al. (2021)

11.2 Organic Carbon and the Transfer of Energy Through Ecosystems

Dissolved organic carbon (DOC) is among the largest reservoirs of organic matter on Earth, and its use and reuse in ecosystems is mediated by microbes. By consuming DOC and incorporating it as biomass in the microbial loop (Azam et al. 1983; Hollibaugh and Azam 1983), microbes serve as a trophic link that transfers organic carbon to higher trophic levels. Predation of microbes by benthic suspension feeders and nanoflagellate planktonic protists mediates this transfer and prevents organic carbon from accumulating in the microbial food web. On coral reefs, the DOC pool is continuously replenished by benthic primary production, whose rates range from 256 to 1696 mmol C m^{-2} d^{-1} and compare to those of tropical rain forests (Odum and Odum 1955; Crossland et al. 1991; Williams et al. 2004; Cardini et al. 2016). Benthic primary producers, including corals, algae, and crustose coralline algae, differ in their rates of DOC production and release, and the relative proportions of each group on a reef benthos can have a substantial influence on the quantity and composition of reef DOC available for microbial consumption (Cardini et al. 2016; reviewed in Nelson et al. 2013, 2023; Wegley Kelly et al. 2022). For instance, whereas corals invest up to 50 to 80% of the photosynthetically fixed carbon from their endosymbionts into growth and calcification (Hatcher 1988; Falkowski et al. 1993; Houlbrèque and Ferrier-Pagès 2009; Tremblay et al. 2012a, b), algae release as much as 60% of their fixed carbon into the surrounding seawater (Jokiel and Morrissey 1986; Crossland 1987; Cheshire et al. 1996). High release rates of DOC by fleshy algae enrich overlying reef water with a high energy food source for microbes, increasing e$^-$DAR and serving as the first step in a regime of degradative microbial phase shifts that reinforce DDAM (reviewed in Silveira et al. 2017). Increasing e$^-$DAR drives microbial expansion by (1) selecting for microbial communities dominated by super-heterotrophs, (2) shifting microbial carbon metabolism to low efficiency strategies that increase microbial biomass, (3) facilitating shifts in viral infection strategies that remove top-down control on microbial expansion, and (4) contributing to the rise of pathogens.

11.2.1 Microbial Community Structure and Biomass

Coral and macroalgae differentially shape the taxonomic structure of reef-associated microbial communities through the release of DOC (Barott et al. 2011; Hester et al. 2016; Walter et al. 2016). Coral-derived DOC, in the form of mucus, is rich in lipids and proteins and selects for mainly oligotrophic microbial taxa (Ducklow and Mitchell 1979; Meikle et al. 1988; Haas and Wild 2010; Nelson et al. 2013). Reefs with high coral cover support highly diverse microbial communities enriched in *Synechococcus* and taxa within the Alphaproteobacteria such as Sphingomonadales, Rhodobacterales, and SAR11 (Nelson et al. 2013; McNally et al. 2017). In contrast, macroalgae release up to seven times as much DOC as coral, and exudates rich in labile carbohydrates and depleted in organic nutrients stimulate rapid consumption by microbial heterotrophs (Ducklow and Mitchell 1979; Meikle et al. 1988; Haas and Wild 2010; Nelson et al. 2013; Wegley Kelly et al. 2022). Algal-dominated reefs support low diversity, copiotrophic microbial communities enriched in Bacteroidetes, Betaproteobacteria, and Gammaproteobacteria such as Alteromonadales, Pseudomonadales, and Vibrionales (Nelson et al. 2013; Haas et al. 2016; Zaneveld et al. 2016; Meirelles et al. 2018). Reef benthic cover of coral and macroalgae, and thus the quantity and composition of DOC available to reef microbes, is consistently one of the strongest predictors of microbial community taxonomic composition in overlying reef water (Dinsdale et al. 2008; Haas et al. 2016; Kelly et al. 2014; reviewed in Silveira et al. 2017).

The enrichment of reefs with macroalgal DOC also stimulates the growth and increased abundances of physically larger microbes. A survey of microbial abundance and cell size on coral reefs across the Pacific Ocean found that degraded, eutrophied reefs supported higher microbial densities and total community biomass relative to coral-dominated sites (McDole et al. 2012). This increase in microbial biomass can be partially explained by the shift in microbial taxonomic composition on algal-dominated reefs, as microbial "super-heterotrophs" have higher growth rates, larger genomes, and are larger in size than oligotrophic taxa (McDole et al. 2012; Haas et al. 2016). The high free energy content of macroalgal exudates, which contain a high proportion of reduced sugars and are depleted in organic nutrients (Kelly et al. 2022), increases the carrying capacity of the ecosystem, supporting higher microbial abundances that increase total community biomass. Considering nearly 100% of available metabolic energy in the water column on degraded reefs is allocated to the microbes, this small increase in total microbial biomass represents a large shift in the distribution of reef energy (DeLong et al. 2010; McDole et al. 2012; Haas et al. 2016). Yet, microbial abundances and taxon-dependent size differences are not alone sufficient to explain the increase in microbial biomass at degraded sites.

11.2.2 Microbial Metabolism

A shift in microbial carbon metabolism towards anabolic pathways is the primary mechanism by which microbial biomass is accumulated on degrading reefs (Haas et al. 2016; Somera et al. 2016). Metabolic shifts were first observed on coral reefs through changes in bacterial growth efficiency (BGE), or the amount of bacterial biomass produced per unit of organic carbon consumed (Haas et al. 2011, 2013a, b). BGE on coral exudates can exceed 18% but is reduced to as low as 6% on algal exudates (Nelson et al. 2013), indicating a decoupling between catabolic (energy-producing) and anabolic (energy-consuming) processes among microbial communities (Del Giorgio and Cole 1998; Carlson et al. 2007). Using metagenomics, Haas et al., showed that microbial communities at coral-dominated sites encode genes for the energy efficient Embden-Meyerhof-Parnas (EMP) glycolytic pathway but shift to the less efficient Entner-Doudoroff (ED) and Pentose Phosphate (PP) pathways as benthic algal cover increases (Haas et al. 2016; Silveira et al. 2019). These

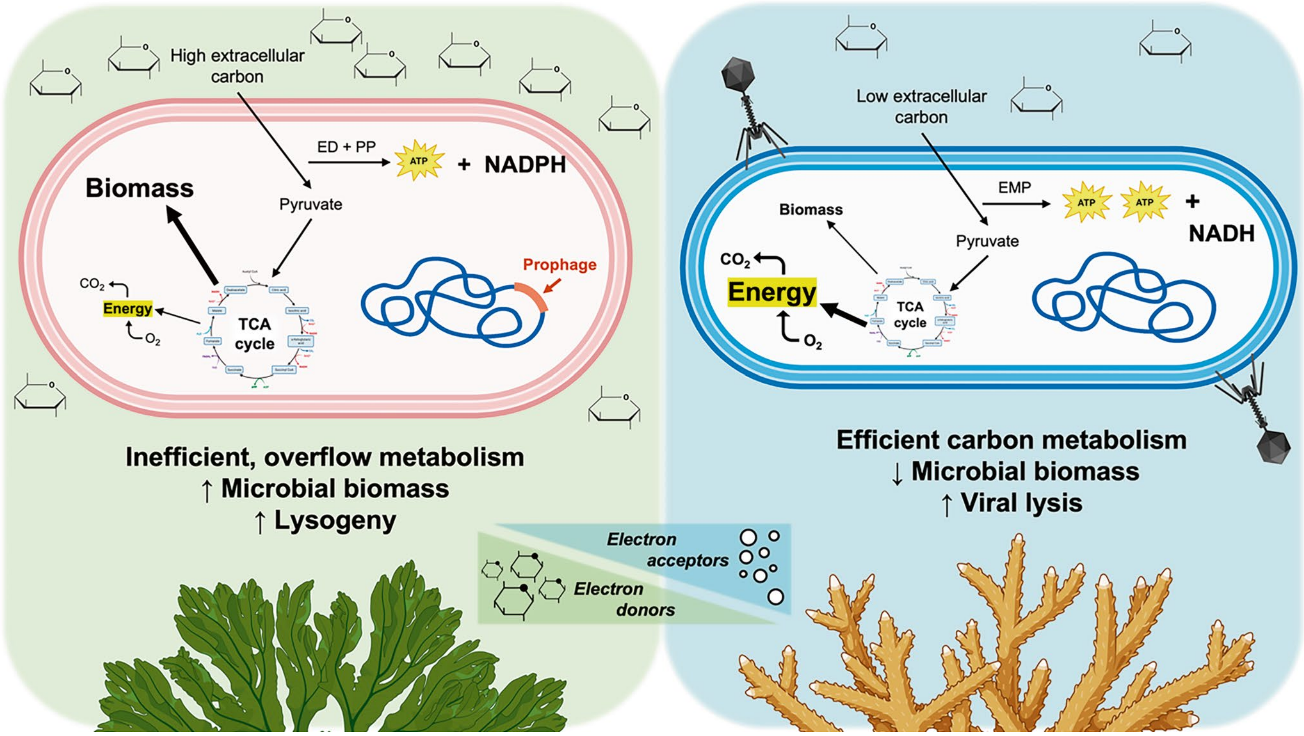

Fig. 11.3 The role of e-DAR in determining microbial community structure and function on coral reefs. (Left Panel) At high e-DAR (abundant electron donors relative to acceptors, i.e., algal-dominated reefs), microbes preferentially use the fast, but inefficient Entner Doudoroff (ED) and Pentose Phosphate (PP) pathways for metabolizing carbon substrates. Shifts to overflow metabolism result in incomplete carbon oxidation and shunt excess carbon into biosynthesis, increasing microbial biomass. High concentrations of NADPH and relatively less ATP in the intracellular environment favor viral integration into host genomes as prophages. (Right Panel) At low e-DAR (abundant electron acceptors relative to donors, i.e., coral-dominated reefs), microbes preferentially use the energy efficient Embden-Meyerhof-Parnas (EMP) pathway for metabolizing carbon substrates, which results in full oxidation of carbon substrates to CO_2. High production of ATP and NADH are used for maintenance costs and favor viral lysis, which serves as a trophic control on microbial community growth

measurements of BGE and genomic indicators of microbial metabolism suggest that microbes respond to a surplus of labile carbon by switching from highly efficient metabolic pathways that maximize the use of limited carbon substrates to less efficient, faster pathways in a canonical yield-to-power switch (Flamholz et al. 2013; Lipson 2015; Haas et al. 2016; Roach et al. 2017). The canonical EMP route generates more ATP and NADH, driving metabolic pathways towards oxidative phosphorylation and the complete oxidation of the carbon substrate to CO_2 (Fig. 11.3, Russell and Cook 1995; Pollak et al. 2007; Spaans et al. 2015). This strategy is well-suited to environments with limited organic carbon supply and abundant oxygen, such as oligotrophic coral reefs and the open ocean. Microbes in these systems devote available energy towards maintenance costs, preserving cellular function and integrity (De Mattos and Neijssel 1997; Hoehler 2004).

Microbes growing on the abundant labile carbon in macroalgal exudates preferentially utilize the alternative ED and PP glycolytic pathways, which produce less ATP and more NADPH (Fig. 11.3, Russell and Cook 1995). Abundant NADPH and depleted ATP drive pathways related to overflow metabolism, which shunt excess organic carbon into biosynthesis as opposed to being oxidized to CO_2 (Basan et al. 2015; reviewed in Russell and Cook 1995). This switch enables microbes in eutrophic environments to metabolize the excess organic carbon faster, at the expense of metabolic efficiency (Stettner and Segrè 2013; Lipson 2015). Because microbes utilizing overflow metabolism do not fully oxidize the available carbon substrate, they consume less oxygen relative to organic carbon and store a larger fraction of the available carbon as biomass. This reduced oxygen consumption per unit carbon would suggest an increase in available oxygen relative to organic carbon in algae-stimulated microbial communities, or a decrease in e^-DAR. However, enhanced rates of respiration and DOC consumption coupled with increased microbial abundance and community biomass ensure a net depletion of oxygen relative to DOC, increasing e^-DAR.

11.2.3 Viral Predation

Increased microbial abundances and metabolic switching at high e^-DAR modulates microbial interactions with viral predators which result in the loss of viral predation pressure on reefs (Fig. 11.3). Viruses utilize two dominant modes of infection: a lytic strategy which terminates in lysis of the bacterial host, or a dormant lysogenic strategy in which viruses establish a long-term relationship with the bacterial host by integrating into the host genome as a prophage (reviewed in Howard-Varona et al. 2017). Coral-dominated reefs support high viral lytic production and relatively lower microbial abundances (Payet et al. 2014; Silveira et al. 2015), implicating viral lysis as a major trophic control of reef microbes (Thurber et al. 2017). The release of bacterial cell contents through viral lytic predation in the so-called viral shunt reduces the transfer of OM to higher trophic levels and instead recycles bacterial carbon back to the DOC pool, where it enhances primary productivity in planktonic food webs (Suttle 2005, 2007). In contrast, high bacterial abundances on algal-dominated reefs are accompanied by an increased frequency of lysogeny and the abundance of temperate phages (Knowles et al. 2016), which act to enhance microbial loop activity and cause OM to accumulate in microbial biomass.

The lysis-lysogeny decision is driven primarily by the metabolic state of the host cell, in which high energy conditions inside the cell (high ATP) tend to favor lysis and low energy conditions inside the cell (low ATP) tend to favor lysogeny (Echols 1986; Kobiler et al. 2004; Laganenka et al. 2019). At the ecosystem level, the energy state of host cells is related to microbial density, with low intracellular ATP conditions, and therefore lysogeny, more common at high and low host densities (Fig. 11.2, Knowles et al. 2016, 2017; reviewed in Silveira et al. 2021). When resource poor conditions support low host densities ($>10^4$ mL^{-1}), such as in the deep ocean, slow-growing, starved, and ATP-depleted microbes favor lysogeny in the Refugium Hypothesis. At intermediate bacterial densities (10^5–10^6 mL^{-1}), such as those found in the open ocean, higher viral-bacterial encounter rates and high intracellular ATP concentrations favor viral lysis in the Kill-the-Winner strategy (Box 11.1, Cheng et al. 1988; Thingstad 2000; Thingstad et al. 2014). However, at high host densities ($>10^6$ mL^{-1}) in microbialized systems, microbes using anabolic pathways with low ATP yield and increased production of NADPH create an intracellular environment favoring the buildup of phage repressors, which stimulate new lysogenic infections and maintain existing prophages (Silveira et al. 2021). The increased frequency of lysogeny at high host densities is referred to as the Piggyback-the-Winner hypothesis and has been observed in ecosystems ranging from aquatic and terrestrial systems to holobionts in both virus-to-microbe ratios (Box 11.1) and metagenomes (Knowles et al. 2016; Touchon et al. 2016). The implications of the lysis-lysogeny decision on reef biogeochemistry are substantial, with viral lysis removing up to half of bacterial standing stock each day in healthy reef systems (Suttle 2007; Payet et al. 2014; Bouvy et al. 2015; Breitbart et al. 2018) and acting as a primary top-down control on microbialization (McDole et al. 2012; Silveira et al. 2023). In contrast, lysogeny facilitates microbial community persistence and expansion on reefs, and contributes to the death of reef macrofauna through the rise of pathogens.

11.2.4 Rise of Pathogens

Lysogeny reinforces microbial dominance on degrading reefs by enhancing bacterial fitness and removing top-down predatory control by other viruses and by protist grazers, accelerating the positive feedback loop of microbial biomass accumulation (Silveira et al. 2017). Prophages encode auxiliary metabolic genes (AMGs) that modulate existing host functions or confer new abilities that improve the chances of survival of the virus-host pair (Canchaya et al. 2003; Feiner et al. 2015; Howard-Varona et al. 2017). Phage-encoded virulence factors enable microbes to recognize and invade metazoan hosts, and are commonly involved in eukaryotic host attachment, invasion, immune system evasion, and toxin production (Silveira et al. 2020). These genes enable microbes to expand their niche, as well as to evade predation by single-celled protistan grazers, which contribute to up to 50% of bacterial predation and transfer bacterial carbon to higher trophic levels (Sherr and Sherr 2002). Reefs with high microbial densities display an increased abundance of phage-encoded virulence genes (Brüssow et al. 2004; Knowles et al. 2016; Cárdenas et al. 2018; Silveira et al. 2020), providing a mechanism to explain the increased abundance of microbial pathogens on degrading reefs. With phage-mediated enhancements in fitness and a suite of virulence factors, reef-associated microbes become agents of disease, contributing to coral death. Further, prophages protect their hosts against infection and lytic predation by other viruses through a defense strategy known as superinfection exclusion, facilitating persistence of the lysogen (Sternberg et al. 1978; Bondy-Denomy et al. 2016; Dedrick et al. 2017). This loss of predatory control over microbial communities by viruses and protists serves as the proverbial “nail in the coffin” for reefs, accelerating transitions to higher microbial energy use.

In summary, changes in e^{-}DAR represent a substantial shift in an ecosystem’s carbon budget and are linked to the physical structure and function of coral reefs. In coral-dominated systems, carbon fixed in photosynthesis provides the energy required for corals to build complex and foundational habitats through calcification. In contrast, the fate of algal-derived carbon does not contribute to an ecosystem-building process (Hughes et al. 2007), but instead feeds into the microbial food web. As a result of shifts in microbial community structure and metabolism, a large fraction of this surplus carbon is stored in microbial biomass (Haas et al. 2016). Temperate viruses, sensing the shifted energetic environment within microbial hosts, opt to integrate into host genomes, and carry virulence genes to enhance host fitness and evade predation (Knowles et al. 2016; Silveira et al. 2023). The loss of controls on microbial growth prevents the transfer of microbially-incorporated carbon back up the trophic web, further accumulating ecosystem energy in the microbial food web.

11.3 Deoxygenation in Aquatic Systems: A Microbial Matter

Oxygen is a primary electron acceptor driving aerobic respiration in nearly all marine organisms, and its abundance is regulated primarily by metabolism (reviewed in Nelson and Altieri 2019). While photosynthesis enriches water with oxygen, respiration depletes it, and influxes of organic matter (OM) that stimulate microbial respiration can result in imbalances in net metabolism that cause deoxygenation. OM is not evenly distributed across ecosystems: it is incorporated in microbial biomass during growth, transferred up trophic levels, released in pulses as organisms die, and accumulated in sediments and at hydrological and geomorphological boundaries. Sites of OM accumulation are hotspots of microbial activity and the resulting deoxygenation drives shifts in ecosystem trophic structure, energy utilization, and biogeochemical cycling. By limiting aerobic respiration, deoxygenation constrains an ecosystem’s energetic potential, because the alternative energy producing pathways and electron acceptors associated with anoxic conditions yield less energy (Falkowski et al. 2008; Wright et al. 2012), and explicitly favor microbial communities capable of sustained anaerobic metabolism over macrobes. As such, ecosystem energy previously allocated to expensive macroecological interactions, such as predation and competition, is transferred to the microbes as ecosystems become deoxygenated (Fig. 11.6).

Microbial degradation of OM depletes electron acceptors available for aerobic respiration, increasing e^{-}DAR and reinforcing transitions to higher microbial energy use (i.e., microbialization). This section emphasizes the relationship between OM, microbes, and deoxygenation in aquatic systems. On coral reefs, sporadic and natural influxes of OM can cause (1) local and acute hypoxic episodes, but enhanced OM loading to coastal ecosystems and resulting microbial community responses have sparked a paradigm of (2) chronic deoxygenation on coral reefs. In addition to consuming oxygen, enhanced respiration of OM decreases seawater pH locally through the production of CO_2, causing (3) acidification and metabolic dissolution that further compromise the growth and survival of reef macrobes, particularly those of calcifiers. Ecosystems at the extremes of OM accumulation, deoxygenation, and acidification may become (4) permanently microbialized, and microbial processes therein play a key role in global biogeochemical cycling. However, expansion of these zones due to (5) climate change and globally increased OM inputs have increased the incidence and scale of coastal “dead zones,” and will intensify microbialization processes to the detriment of coastal ecosystem health and productivity.

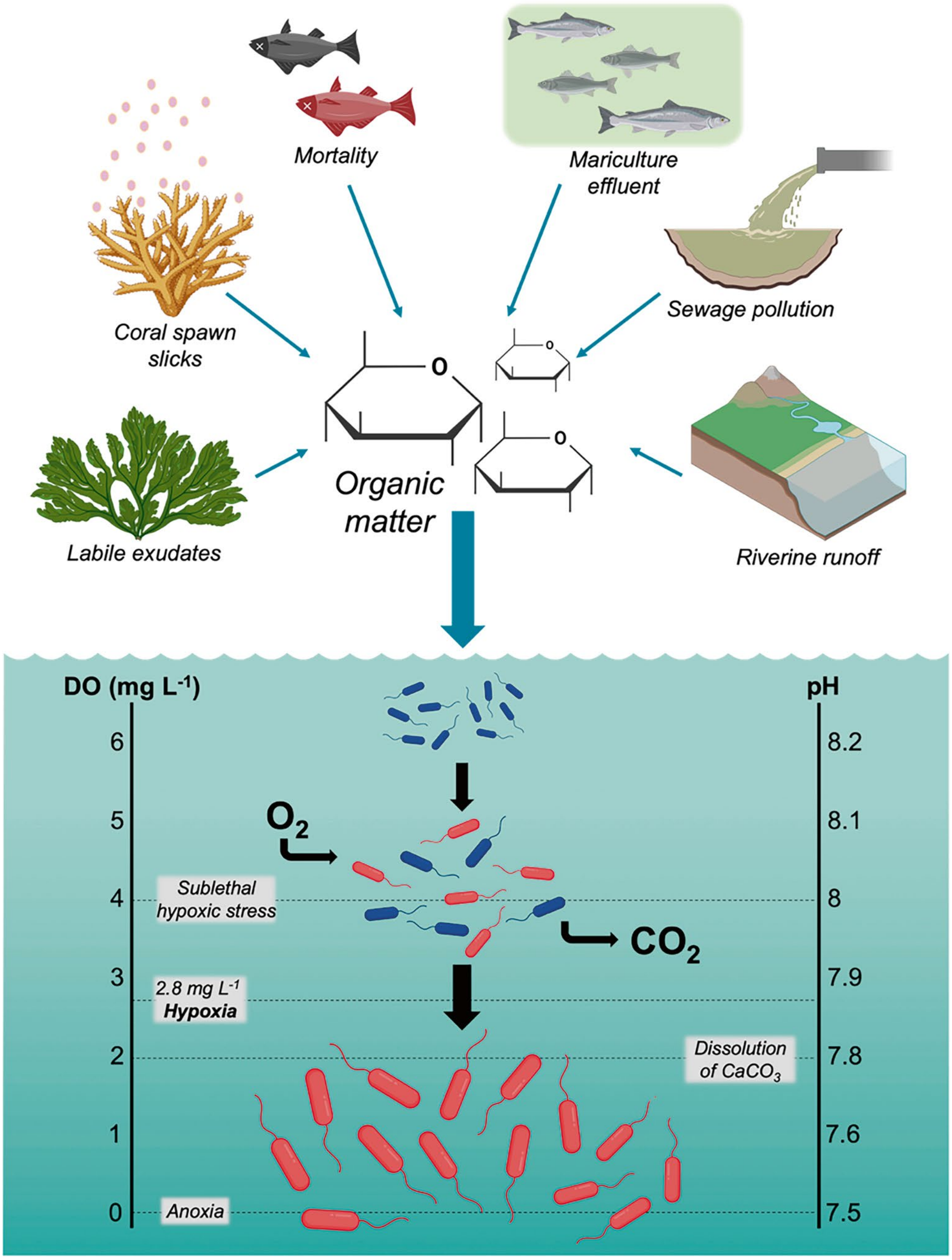

Fig. 11.4 Organic matter inputs as a driver of deoxygenation and acidification in aquatic systems. Microbial degradation of (1) labile macroalgae exudates, (2) larval biomass following coral spawning events, (3) dead and decaying reef organisms, (4) nutrient-rich effluent from fisheries, (5) anthropogenic wastewater, and (6) terrestrial and agricultural runoff from river discharge can reduce local oxygen concentrations and pH to lethal levels for reef macrofauna. Persistent OM inputs, combined with local geomorphological characteristics which prevent mixing with more oxygenated waters, can result in long-term or permanent conditions of hypoxia and reduce the aragonite saturation state below thresholds necessary for calcification processes

11.3.1 Acute Deoxygenation on Coral Reefs

Coral reefs have a net metabolic balance close to zero (Crossland et al. 1991), with rates of high primary production met with equally high rates of consumption, decomposition, and recycling. Despite this relative balance between autotrophy and heterotrophy (Alldredge et al. 2013; Naumann et al. 2013; Rix et al. 2015), diel and seasonal fluctuations in physical factors, nutrient inputs, and biogeochemistry can temporarily shift reef metabolism in favor of heterotrophy. Periods of net heterotrophy are commonly driven by an accumulation of OM over relatively short time scales or in shallow, stratified, or confined water masses. Rapid microbial decomposition of this accumulated OM can result in the formation of suboxic conditions at the coral reef benthos and throughout the water column that can last for several days (Fig. 11.4, Best et al. 2007). Episodic microbial deoxygenation on coral reefs has been documented following coral larval slicks (Glud et al. 2008; Patten et al. 2008; Wild et al. 2008), extreme tidal fluctuations (Simpson et al. 1993; Villanueva et al. 2005; Hobbs and Macrae 2012), phytoplankton blooms resulting from nutrient-rich terrestrial runoff (reviewed in Fabricius 2005; Kealoha et al. 2020), sewage pollution (Smith et al. 1981; Jokiel et al. 1993), mariculture effluent (Loya 2004; Villanueva et al. 2005), and coastal upwelling (Genin et al. 1995; Laboy-Nieves et al. 2001).

Hydrological and geomorphological characteristics of reefs can enhance their susceptibility to acute suboxic events by facilitating OM accumulation and reducing the replenishment of oxygen depleted by microbial respiration. Shallow, semi-enclosed sites with restricted water flow such as lagoons (Camp et al. 2017), reef flats (Guadayol et al. 2014), atolls (Andréfouët et al. 2015), embayments, and tide pools routinely experience periods of suboxia (<2–3 mg/L O_2), which can become hypoxic during tidal and seasonal warming events that increase basal rates of respiration and microbial oxygen demand (Meire et al. 2013). Risk of deoxygenation is further compounded at sites in close proximity to terrestrial inputs and with limited flushing from the surrounding ocean (Kraines et al. 1996; Diaz and Rosenberg 2008; Andréfouët et al. 2015; Altieri et al. 2021). Suboxic and hypoxic events are more common during calm weather, when light winds, reduced current speeds, and low swell cause the water column to stratify, reducing mixing and the transfer of oxygenated surface water to deeper layers (Simpson et al. 1993; Hobbs and Macrae 2012; reviewed in Gobler and Baumann 2016).

Acute microbial deoxygenation is an agent of stress and mortality for reef macrobes and can impact local benthic community structure. Reef organisms display a wide range of tolerance to suboxic conditions and are accustomed to some natural variation in dissolved oxygen (DO) concentrations due to diel and seasonal fluctuations (Altieri et al. 2021; Diaz and Rosenberg 1995; reviewed in Nelson and Altieri 2019). Broadly, periodic hypoxia affects marine organisms by altering behavior and immune responses, enhancing susceptibility to disease, and impairing growth and reproduction (reviewed in Breitburg et al. 2018 and Nelson and Altieri 2019). In corals specifically, low oxygen conditions can cause bleaching, tissue loss, DNA damage, and shifts in metabolism, photosynthetic capacity, and calcification rates which compromise coral health and function (see Pezner et al. 2023 and citations therein). Depending on the duration, frequency, and magnitude of the suboxic conditions, many reef organisms can recover from episodes of acute microbial deoxygenation. Johnson et al. (2021) documented the recovery of a coral reef community following a severe, multi-day hypoxic event and found that while water column microbial communities rebounded to pre-hypoxic states within days, changes to benthic communities persisted for more than a year, with marked losses in coral cover and invertebrate diversity (Johnson et al. 2021). These findings indicate a decoupling in ecological trajectories between microbes and macrobes following disturbance (Johnson et al. 2021). Due to a combination of global climate change and increased OM inputs to coastal ecosystems, acute deoxygenation events are becoming more frequent, severe, and longer in duration on coral reefs (Fig. 11.5, Breitburg et al. 2018; Alteri et al. 2019), with 15% of coral reefs estimated to be at an elevated risk of hypoxia (Altieri et al. 2017; Hughes et al. 2020). Increasing e^{-}DAR on reefs as a result of increasing OM inputs and active oxygen loss will contribute to a chronic paradigm of deoxygenation challenging the recovery of degraded reef communities.

11.3.2 Chronic Deoxygenation of Coral Reefs

Reefs under phase shift towards macroalgal dominance are threatened by chronic deoxygenation. Algae-dominated reefs have lower DO standing stocks, with nighttime respiratory drawdown causing DO to approach hypoxia at many sites (Wild et al. 2010; Haas et al. 2013a; Altieri et al. 2021; Pezner et al. 2023). This observation is counter to experimental studies of oxygen production by benthic primary producers, which show turf- and fleshy- macroalgae release up to three times as much oxygen into the surrounding seawater as calcifying organisms (Naumann et al. 2010; Haas et al. 2011; Nelson et al. 2013; Silveira et al. 2019). These findings can be explained by two mechanisms which result in (1) active loss and (2) increased consumption of oxygen on algae-dominated reefs. Silveira et al., described a biophysical mechanism by which photosynthetically produced oxygen supersaturates at the surface of fleshy algae, forming bubbles through heterogeneous nucleation, which, when liberated from the algal surface, are lost to the atmosphere (Fig. 11.5, Odum and Odum 1955; Kraines et al. 1996; Freeman et al. 2018; Silveira et al. 2019). In contrast, 78–90% of the photosynthetic oxygen produced by endosymbiotic microalgae living within coral tissues is provided to the coral host to sustain the energetic demands of respiration and calcification (Al-Horani et al. 2003a, b), thus retaining oxygen within the benthic community. The process of oxygen bubbling, known as ebullition, has been documented in several aquatic systems and is predicted to account for the loss of up to 37%, 21%, and 20% of gross oxygen production in lakes (Koschorreck et al. 2017),

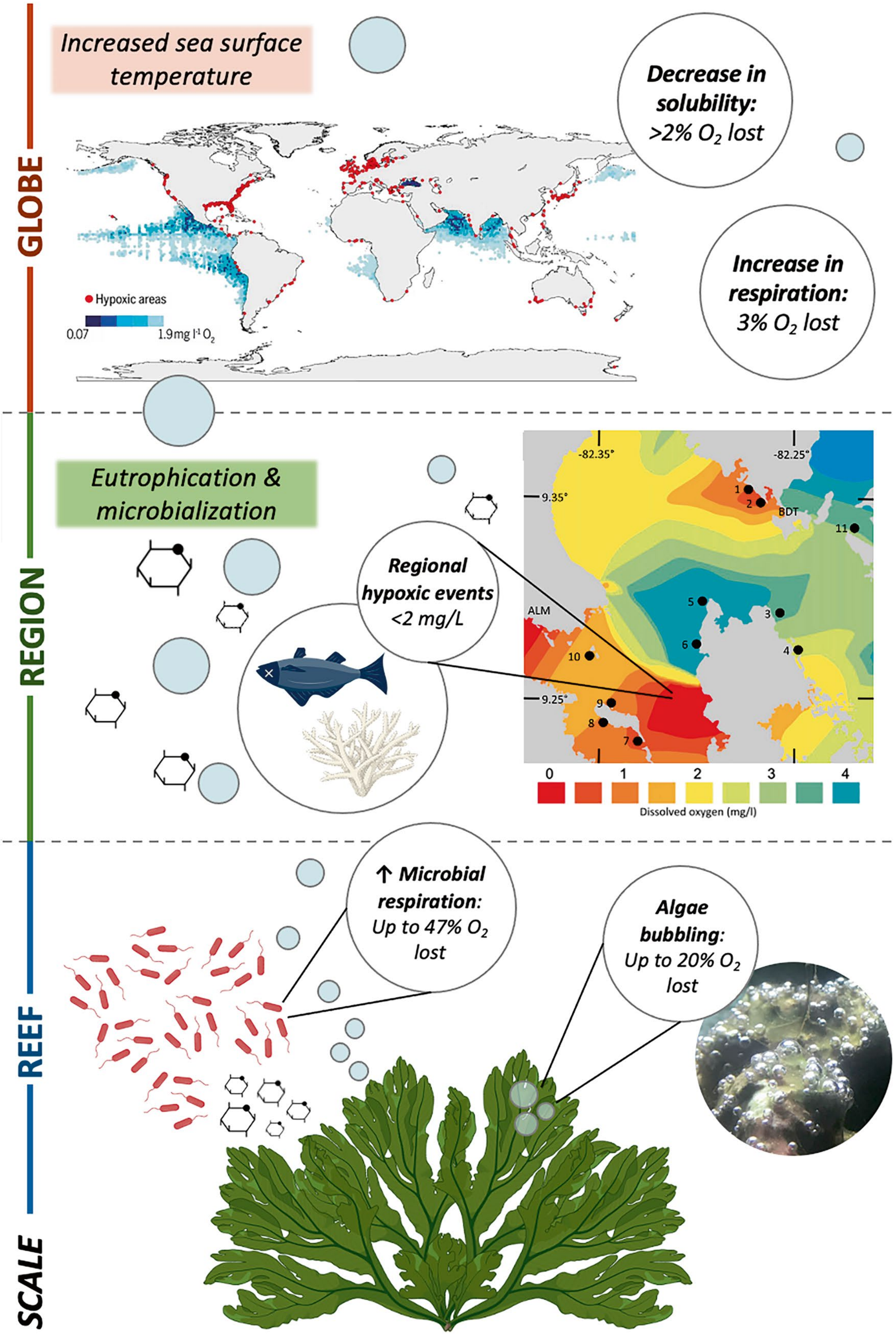

Fig. 11.5 Microbial deoxygenation as a feature of coastal ecosystems spanning multiple scales, driven by anthropogenic inputs of organic matter and climate change. At local reef scales, the overgrowth of fleshy macroalgae can result in the loss of up to 67% of gross oxygen production through ebullition and enhanced microbial respiration of algal-derived organic matter. At regional scales, terrestrial inputs of organic matter and accompanying microbial decomposition can result in suboxic events which kill benthic invertebrates, including corals. At the global scale, increases in sea surface temperature result in global ocean deoxygenation through reduced oxygen solubility and increased respiratory demand of micro- and macro-organisms. World map figure (top panel) adapted from Breitburg et al. (2018). Map of Panama (middle panel) adapted from Altieri et al. (2017)

salt marshes (Howard et al. 2018), and algal-dominated reefs (Silveira et al. 2019), respectively.

By releasing oxygen through ebullition and retaining labile carbon exudates in solution, algae increase e^{-}DAR and create a high energy, low oxygen environment which stimulates microbial heterotrophic metabolism (Haas et al. 2010, 2011; Wild et al. 2010; Nelson et al. 2013; Kelly et al. 2014). The resulting increase in microbial heterotrophy is the second mechanism contributing to oxygen loss on coral reefs: microbialization increases a reef's baseline biological oxygen demand (Fig. 11.5). In mesocosm incubations, microbial communities growing on labile macroalgal exudates had higher respiratory demand and consumed 10 times more oxygen than those growing on coral exudates (Silveira et al. 2019). At the coral-algae interface, this increased microbial growth and oxygen demand can cause suboxic zones which result in coral death (Barott et al. 2009; Gregg et al. 2013; Haas et al. 2013a, b, 2014; Roach et al. 2017). The formation of microbially mediated suboxic zones through the release of labile DOC has been implicated as a major strategy for turf- and fleshy-macroalgae to gain a competitive advantage over corals in the struggle for benthic space. At the scale of a reef, microbial respiration can consume up to 47% of the oxygen produced by benthic primary producers and, together with ebullition, may result in the loss of almost two thirds of gross oxygen production on reefs (Silveira et al. 2019).

11.3.3 Microbial Acidification

The consequences of enhanced microbial heterotrophy during microbialization often focus on depletion of oxygen and overlook the production of carbon dioxide (CO_2), which reduces seawater pH and drives acidification. Open ocean pH is controlled primarily by atmospheric exchange of CO_2, leading to relatively low interannual variation in pH (<0.1 unit) (Caldeira and Wickett 2003). In contrast, pH in highly productive coastal ecosystems is strongly regulated by metabolism and displays diel and seasonal fluctuations up to an order of magnitude higher than open ocean systems, with daily ranges of up to 1 pH unit observed on coral reefs (Borgesa and Gypensb 2010; Hofmann et al. 2011; reviewed in Duarte et al. 2013). Photosynthesis and respiration modify local pH through the consumption and production of CO_2, respectively, and elevated microbial respiration of OM reduces both pH and the availability of carbonate ions (CO_3^{2-}) essential to calcification (Feely et al. 2008; Cai et al. 2011; Wallace et al. 2014). This "metabolic acidification" reduces the saturation state for $CaCO_3$ minerals such as aragonite, negatively affecting $CaCO_3$ production in calcifying organisms and accelerating reef bioerosion and dissolution (Yeakel et al. 2015). In eutrophied coastal areas, seasonal and sometimes daily levels of CO_2, aragonite saturation, and pH already exceed (1) thresholds that are known to reduce growth and survival in marine organisms and (2) predicted extremes in the open ocean due to ocean acidification (Melzner et al. 2013; Wallace et al. 2014). While hypoxia and acidification tend to co-occur following episodes of enhanced microbial respiration, low pH conditions persist longer than hypoxia due to differences in rates of CO_2 and O_2 diffusion and solubility (Wallace et al. 2014). The combined effects of these processes dampen net reef accretion by enhancing metabolic dissolution (Eyre et al. 2014; Cyronak and Eyre 2016), negatively impact the growth and survival of calcifying organisms (Mccoy and Kamenos 2015; Steckbauer et al. 2020), and exacerbate organismal responses to deoxygenation (see for citations Breitburg et al. 2018; Steckbauer et al. 2020), reinforcing transitions from biodiverse, accreting reefs dominated by calcifiers to low diversity, actively dissolving reefs dominated by algae and microbes (Yates et al. 2017).

Metabolic acidification may also enhance positive feedback to higher e^{-}DAR by altering the composition of DOC available for microbial consumption. The DOC pool comprises an immense diversity of chemical compounds whose residence time in seawater is determined by their ability to be degraded by microbes, with highly labile carbon compounds degraded easily on the order of minutes to hours and refractory carbon compounds resisting degradation and persisting in seawater over much longer timescales (Carlson and Ducklow 1996; Carlson et al. 2007). Efforts to balance carbon budgets in terrestrial systems led to the discovery of the priming effect, in which the addition of labile organic carbon compounds induce co-metabolism interactions among microbial communities which enable them to degrade more refractory organic carbon (reviewed in Guenet et al. 2010). The priming effect "diversifies the menu" for microbes, facilitating the consumption of more of the DOC pool and, in marine systems, results in measured values of DOC inventory that are lower than expected given organic carbon inputs (Thingstad et al. 2008; Guenet et al. 2010; Haas et al. 2016). Reduced seawater pH enhances both (1) the production of labile organic carbon sources such as transparent exopolymer particles (TEP) and (2) the net rates of organic carbon loss (Engel et al. 2004; Riebesell et al. 2007), suggesting acidification may enhance the lability of the DOC pool and, through the priming effect, the amount of the DOC pool respired to CO_2. Intensification of the priming effect via increased inputs of CO_2 and OM may therefore serve as a feedback loop that amplifies metabolic deoxygenation and acidification in eutrophied coastal systems.

11.3.4 Microbial Hotspots: Life at the e-DAR Extremes

Where consistently high OM inputs combine with physical features restricting water movement and mixing, ecosystems can become permanently microbialized and commonly experience hypoxic and acidified conditions for extended periods of time or in perpetuity. At these extreme ends of the e-DAR spectrum, ecosystem energy use is dominated by microbes, and low oxygen conditions support microbial processes that are major contributors to global biogeochemical cycles (reviewed in Wright et al. 2012). In oceanic oxygen minimum zones (OMZs), microbial degradation of OM from nutrient-rich, upwelled deepwater and a rain of decaying OM from productive surface waters create near-anoxic conditions that facilitate anaerobic processes normally absent in oxic surface waters (Ulloa et al. 2012). As oxygen is depleted, aerobic respiration is replaced by processes including denitrification, anaerobic ammonium oxidation (annamox), and sulfate reduction, which use nitrate (NO_3^-) and nitrite (NO_2^-), ammonium (NH_4^+), and sulfate (SO_4^{2-}) as alternate electron acceptors to degrade OM, respectively. Remineralization of OM by denitrification and annamox to dinitrogen gas (N_2) in OMZs represents up to 50% of bioavailable (fixed) nitrogen loss in the oceans (Codispoti et al. 2001). Denitrification also produces N_2O, a potent greenhouse gas, and OMZs are estimated to account for at least one third of global N_2O emissions (Codispoti et al. 2001; Wright et al. 2012). In the open ocean, microbialization processes driving OMZs are essential to the remineralization and redistribution of inorganic nutrients in the oceans, facilitate the export of OM from surface waters in the biological carbon pump, and impact atmospheric concentrations of gases affecting the global climate.

Microbial degradation of OM also shapes ecological and biogeochemical landscapes in coastal ecosystems. Estuaries are net heterotrophic systems, with high delivery of labile OM via eutrophied river plumes supplementing internal OM accumulation from high primary production rates (Del Giorgio and Williams 2005; Gobler and Baumann 2016). Persistently hypoxic conditions in sediments and stratified bottom water layers of estuaries can expand to affect the entire water column in warmer, summer months (Soertaert et al. 2006), supporting similar anaerobic OM degradation pathways as in OMZs. High denitrification rates in estuaries reduce the concentration of terrestrially derived organic nitrogen by more than 70%, thus helping to mitigate eutrophication to adjacent oceanic ecosystems and serving as a buffer for globally increased anthropogenic inputs of nitrogen (Barbier et al. 2011; Smyth et al. 2013; Pennino et al. 2016). As a sink for terrigenous N, microbialized estuaries control the flux of nutrients to the oceans and can limit the amount of organic nitrogen available for primary production (Seitzinger 1987; Cornwell et al. 1999). However, global increases in temperature and anthropogenic inputs of OM have overwhelmed the capacity of many estuaries to regulate eutrophication, thus expanding microbialization to the coastal ocean and altering global biogeochemical cycles.

11.3.5 Global Changes and Dead Zones

Global changes in climate patterns and ocean conditions will exacerbate and amplify the effects of microbial deoxygenation and acidification in coastal environments (Breitburg et al. 2018; Hughes et al. 2020). Dissolved oxygen concentrations are in decline across global aquatic ecosystems: the open ocean has lost more than 2% of its oxygen content in the past 50 years (Schmidtko et al. 2017) and is expected to lose an additional 3–5% by 2100 (Bopp et al. 2013; Pezner et al. 2023). Oxygen losses are more pronounced in the coastal ocean due to close proximity to terrestrial OM inputs and increased warming of shallower water over continental shelves (Gilbert et al. 2010). Increased precipitation due to ocean warming is enhancing riverine discharge to coastal ecosystems (Justić et al. 1996; Fabricius 2005; Solomon 2007), compounding already considerable OM inputs from anthropogenic activities. Globally increased sea surface temperatures (SST) both reduce oxygen solubility in seawater and increase organismal metabolic rates (Brown et al. 2004; reviewed in Keeling et al. 2010; Vaquer-Sunyer et al. 2012), thus increasing biological oxygen demand while simultaneously reducing its availability. Indeed, hypoxia and acidification at eutrophied sites reach peak highs during warmer summer months, as rapid microbial respiration rates consume oxygen and produce CO_2 faster than they can be replenished and exported, respectively (Wallace et al. 2014).

As warm, fresh water is less dense than cold and salty water, rising SST and increased precipitation act to increase water column stratification of the coastal ocean (Keeling et al. 2010). Stratification isolates deeper water layers from oxygenated surface waters, preventing mixing that would otherwise replenish oxygen consumed by microbial degradation of OM (Sotto et al. 2014). Reduced oxygen resupply to the ocean interior owing to increased thermal stratification has caused open ocean OMZ suboxic boundaries to expand into shallower depths (Whitney et al. 2007), causing habitat compression for pelagic species. By increasing the strength of offshore winds, ocean warming is also increasing coastal upwelling, resulting in the expansion of coastal OMZ onto continental shelves (Stramma et al. 2008, 2010). This "shoaling" of OMZs transports low-oxygen, acidified water to coastal ecosystems and can result in major losses to benthic macrofauna (Chan et al. 2008; Feely et al. 2008; Sydeman et al. 2014). Hypoxia-induced mass mortality of macrobes then provides a rich source of OM for microbial decomposition, creating a feedback loop in which eukaryotic secondary production is vastly reduced and virtually all ecosystem OM is remineralized by the microbes (Diaz and Rosenberg 2008). Collectively, these factors are increasing microbialization in coastal environments by creating OM rich, oxygen depleted, and poorly mixed zones dominated by microbial processes and hostile to macrobial life.

Ecosystems in which severe, prolonged suboxic conditions cause mass mortality or migration of macrobes are known as dead zones (Fig. 11.5, Diaz and Rosenberg 1995; Rabalais et al. 2002), representing the extreme end of the microbialization regime. Anthropogenic OM inputs and climate change have increased the incidence and severity of dead zones in temperate and tropical ecosystems (Fig. 11.5, Diaz and Rosenberg 2008; Rabalais et al. 2014; Altieri and Gedan 2015; Breitburg et al. 2018), with major consequences to coastal fisheries (Diaz and Rosenberg 2008) and ecosystem services. Ecosystem models of hypoxia show that in oxygenated conditions, up to 75% of the energy produced via primary production is allocated to mobile predators (Diaz and Rosenberg 2008), while under conditions of hypoxia, energy is diverted into microbial pathways and away from higher trophic levels (Pearson and Rosenberg 1992; Baird et al. 2004). Yet, despite drastic reductions in macrofaunal biomass and diversity, dead zones are hotspots of microbial life and activity. Globally increased coastal dead zones are expected to impact biogeochemical cycles in similar ways to other highly microbialized habitats, potentially by enhancing losses of bioavailable N and increasing production of greenhouse gases, including N_2O and methane, that impact global climate.

11.4 Coral Reef Arks and the Reduction of e^-DAR

Coral reef microbialization is a global phenomenon and on many reefs has progressed to a stage at which natural recovery processes will not be sufficient to reinstate reef functions, even in the absence of continued anthropogenic impact. At these sites, active and targeted interventions will be necessary to restore and reshape reef ecosystems to the point of self-sufficiency. Early coral reef restoration efforts adopted techniques from forest restoration to create a marine silviculture paradigm known as “coral gardening” (Guzmán 1991; Rinkevich 1995; Epstein et al. 2003), which despite limited efficacy remains a leading practice used today. Current restoration interventions center primarily around the propagation and active translocation of corals to denuded sites, the artificial augmentation of reef three-dimensional framework, and the enhancement of coral sexual reproduction through larval rearing and dispersal (Rinkevich 2019; Boström-Einarsson et al. 2020; Randall et al. 2020; Higgins et al. 2022). Yet, efforts to restore coral reef function and reinstate valuable ecosystem services have not achieved much success (Boström-Einarsson et al. 2020).

11.4.1 How to Restore a Reef?

In this chapter, we have provided evidence that coral reef microbialization is initiated by the loss of predation pressure by fish and viruses and mediated by a change in reef biochemistry through increased e^-DAR (Fig. 11.6). Solutions for restoring reefs may involve combatting these processes by (1) reinstating fish and viral predation pressure or (2) reducing e^-DAR. Both can be addressed in part through active management: enforcement of fishing regulations can reduce local overfishing (Hilborn et al. 2020), and improved methods of wastewater treatment can reduce anthropogenic OM inputs to marine ecosystems (Smith et al. 1981; Kemp et al. 2009). Indeed, active management of nutrient and organic carbon inputs has reduced microbial biological oxygen demand, reestablished oxic conditions, and eliminated dead zones from several coastal and aquatic ecosystems (Diaz and Rosenberg 2008; Kemp et al. 2009). Well-designed and enforced fishing regulations can contribute to the recovery of reef fish populations (Di Franco et al. 2016), which increase coral cover and slow phase shifts to macroalgal states (Hughes et al. 2007). No such methods exist yet for reinstating viral predatory control over microbes, though the enhancement of lytic production and induction among environmental viruses represents a fruitful avenue for research. Engineering solutions have also been proposed to combat deoxygenation, typically involving mechanisms which enhance vertical and horizontal mixing of the water column or resupply oxygen via mechanical air bubbling (Stigebrandt and Gustafsson 2007; Conley et al. 2009), but none have yet been brought to scale.

11.4.2 Reducing e^-DAR Using Coral Arks

Active restoration interventions on coral reefs will benefit from integrating the above goals of reinstating predation pressure and reducing e^-DAR into management plans. Locally reducing e^-DAR on reefs may be achieved simply by moving vertically out of the reef boundary layer. Changes in e^-DAR are most pronounced at the benthic interface, where the concentration of organic carbon exuded by primary producers, microbial activity, and oxygen consumption are at a maximum. Reef e^-DAR is therefore highest at the reef-water interface and decreases with distance from the benthos, suggesting that biochemical conditions may be improved by relocating a portion of the reef community from the benthos

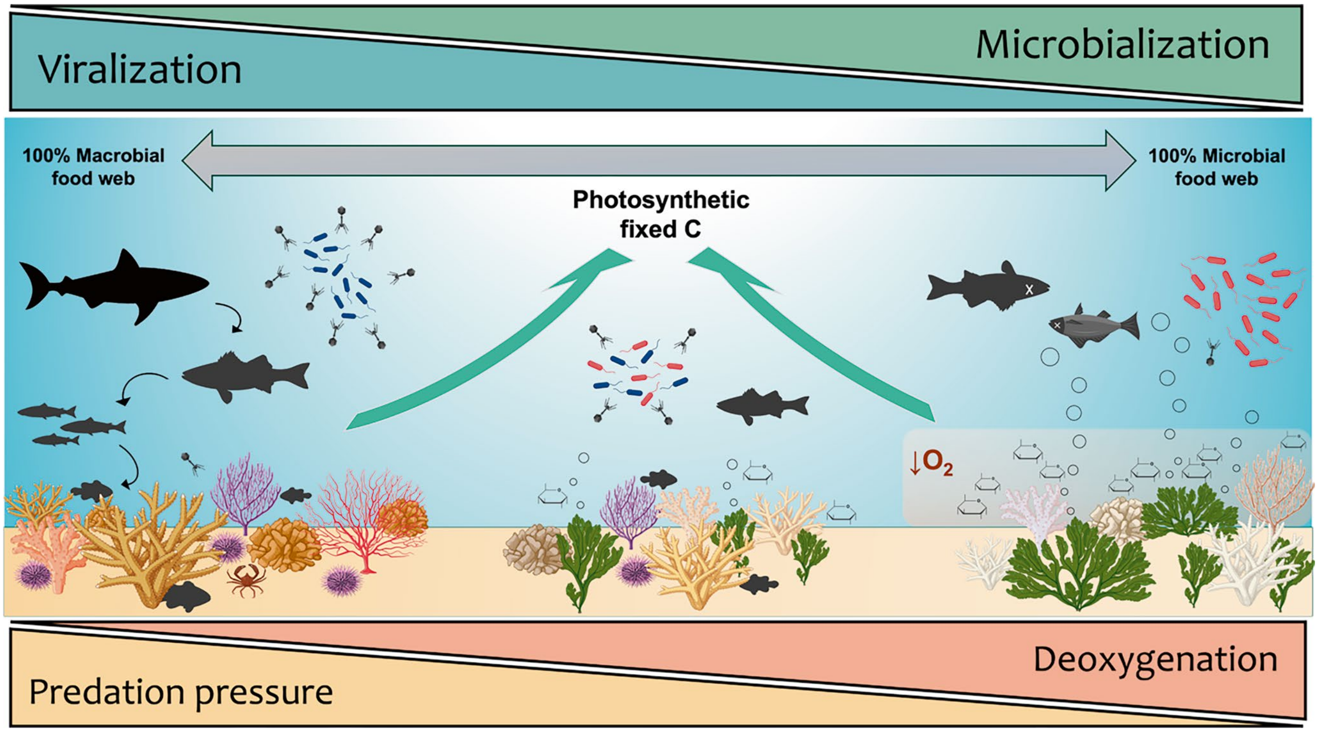

Fig. 11.6 Viralization vs microbialization on coral reefs. On viralized reefs dominated by corals, predation pressure by fish and viruses transfers photosynthetically fixed carbon up to higher trophic levels, maintaining up to 100% of ecosystem energy in the macrobial food web. On microbialized reefs dominated by algae, macroalgal carbon is fed directly into the microbial food web, diverting ecosystem energy away from higher trophic levels. Deoxygenation from algal oxygen bubbling and microbial respiration kill reef macrobes and reinforce microbial dominance. Here, blue microbes represent beneficial or neutral taxa, while red microbes represent copiotrophs and potential pathogens

to the overlying water column. Baer et al. (2023) demonstrated the use of a seafloor-tethered, midwater platform called Coral Reef Arks to support the growth and propagation of coral reef biodiversity (Baer et al. 2023). Survival rates of translocated corals on Coral Arks after one year were three times higher than for corals translocated to nearby denuded seafloor sites. The midwater Coral Arks environment displayed higher dissolved oxygen concentrations, flow speeds, virus-to-microbe ratios (VMRs), and lower DOC concentrations relative to the seafloor control sites (Fig. 11.7), indicating an environment with reduced e$^-$DAR and enhanced viral predation pressure (Baer et al. 2023). Similarly, the Mars Assisted Reef Restoration System (MARRS) elevated corals above the boundary layer and facilitated rapid accretion on “Reef Stars,” leading to reef recovery at highly degraded sites (Williams et al. 2019; Lange et al. 2024).

Population enhancement and restocking of reefs via *in situ* propagation of corals and keystone reef herbivores (i.e., *Diadema antillarum* in the Caribbean) is underway and will benefit from new methods to enhance survival despite deteriorating ecological conditions. Relocating a portion of the reef community to improved conditions in the midwater may be a viable first step for coral reef restoration projects. Escaping the reef boundary layer dampens diel fluctuations in DO and pH which result in nighttime hypoxia and respiratory acidification on algal-dominated reefs. This can be achieved through the use of positively buoyant, fully midwater structures such as Coral Arks, or seafloor-attached structures (such as MARRS’ reef stars) with sufficient height off the benthos to locally reduce e$^-$DAR (Baer et al. 2023; Lange et al. 2024) (Fig. 11.7). Species which play a disproportionate role in maintaining ecosystem functioning, such as corals and grazing invertebrates, are good candidates for translocation to these local biochemical hotspots on an otherwise microbialized benthos. Coral Arks and similar methodologies which enhance reef biochemical conditions while providing habitat for reef macrofauna will support the success of coral restoration efforts and help conserve reef biodiversity while the factors driving global microbialization, namely OM inputs, overfishing, and CO_2 emissions, can be addressed.

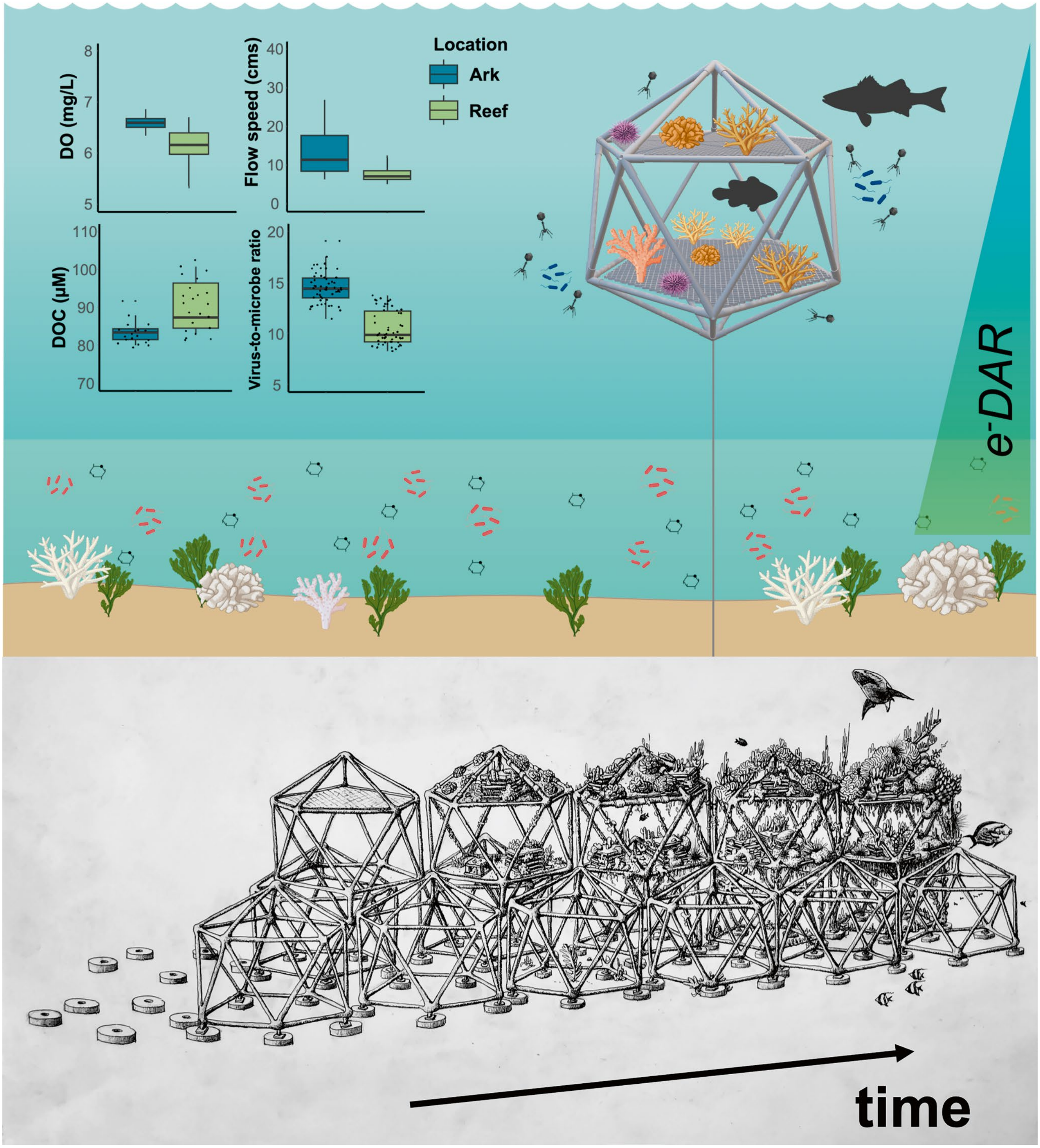

Fig. 11.7 Coral Reef Arks as seafloor-tethered, midwater platforms for recruiting and propagating coral reef biodiversity and reducing microbialization. (Top Panel) By elevating reef communities above the microbialized benthos, Coral Arks provide enhanced oxygen, higher flow speeds, and reduced DOC concentrations (reducing e-DAR overall relative to the benthos). Arks also display higher virus-to-microbe ratios (VMR), indicating enhanced viral lytic control over microbial communities. (Bottom Panel) Arks can be constructed into seafloor-attached, living breakwalls to provide improved habitat for reef species while reinstating reef framework for wave dissipation and coastal protection. Bottom panel illustrated by Ben Darby

11.5 Conclusions

Microbes are the engines that drive Earth's biogeochemical cycles (Falkowski et al. 2008), supporting the global recycling and redistribution of carbon and nutrients across ecosystems. Organic matter represents the energy source feeding microbial engines in aquatic systems and its consumption by microbes, which are in turn consumed by planktonic protists and benthic suspension feeders, transfers this energy up through the trophic web. The high productivity and biodiversity of coral reefs rely on low influxes of OM, efficient trophic transfer of microbially-incorporated carbon via microbial predation, and abundant oxygen. However, global increases in labile OM inputs and decreases in oceanic oxygen content have enhanced processes associated with microbial expansion and diminished those processes integrating microbes into reef macrobial food webs. This microbialization of coral reefs represents a redistribution in ecosystem energy from supporting high macrofaunal biomass, ecological interactions such as predation and symbiosis, and energy intensive processes such as calcification to trophically simplified, oxygen-limited, and eutrophied microbial reactors.

Microbialization is driven by an increase in the ratio of electron donors (i.e., labile organic carbon) to electron acceptors (i.e., oxygen), or e^{-}DAR, in aquatic systems. Increased labile organic carbon causes shifts in microbial community structure that enhance microbial carbon consumption at the expense of metabolic efficiency, reduces connectivity with reef food webs by evading predation, and exacerbates climate change-driven losses in oxygen by increasing biological oxygen demand. Resulting decreases in oxygen, which further increase e^{-}DAR, limit aerobic respiration and divert energy away from macrofauna and into microbial metabolism. This positive feedback between organic matter, microbial metabolism, and deoxygenation reinforces microbial dominance and makes microbialized systems increasingly stable over time, locking resources in the microbial food web. Dead zones represent an extreme outcome of these changes; increases in the incidence and severity of these zones in coastal ecosystems will alter ocean productivity, biodiversity, biogeochemical cycling, and human livelihoods by compromising food security, coastal protection, and other reef ecosystem functions. Efforts to mitigate coral reef microbialization should aim to reduce e^{-}DAR and reinstate predation by herbivorous fish and viruses to control macroalgae and microbes and redirect photosynthetically fixed carbon back up the trophic web. Other means of regulating microbial communities, such as microbial-based therapies, could also contribute to mitigating the microbialization-driven shift towards pathogenic assemblages and support the restoration of aquatic ecosystems.

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Part V

Host-Microbial Interactions as Culprit and Remedy

When Microbial Interactions Go Wrong: Coral Bleaching, Disease, and Dysbiosis

12

Julie L. Meyer, Michael J. Sweet,
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Abstract

Research into an organism's microbiome has become exceedingly popular over the past two decades. These studies have highlighted corals as an excellent model group and illustrated the importance of the host-microbiome relationship within the coral holobiont. Symbioses within the holobiont include a spectrum of relationships ranging from beneficial to harmful. Indeed, the hunt for coral pathogens when a coral bleaches or succumbs to disease is widely researched but comes with its fair share of controversy. In this chapter, we attempt to un-pick the facts from fiction and present a synopsis of the current knowledge on bleaching, disease, dysbiosis, and the role of the microbiome in all this.

Keywords

Bleaching · Disease · Dysbiosis · Koch's postulates
Diagnostics · Microbiome · *Vibrio* · Anaerobes
Pathobiome

12.1 Bacteria and Bleaching

12.1.1 What Started It All

Bleaching in corals has been well documented in the scientific literature and is known to be driven primarily by increases in the sea surface temperature, a response associated with anthropogenic climate change. However, other causes of coral bleaching have also been documented or proposed (van Oppen and Lough 2009). This suggests that a coral can bleach in a multifaceted manner. Most notable for this book is the bleaching of *Oculina patagonica* by the bacterial pathogen *V. shilonii* [formerly *Vibrio shiloi*] (Kushmaro et al. 2001). At the time, this was developed into a 'model system' (of sorts)—one aimed at improving our understanding of how pathogens like *V. shilonii* interact with their host (Rosenberg and Falkovitz 2004). Rosenberg and Falkovitz highlighted that the bacteria could adhere to beta-galactoside-containing receptors in the coral mucus, penetrate the epithelial cells, differentiate into a viable-but-not-culturable state (VBNC), multiply, and then produce a proline-rich peptide toxin that inhibits photosynthesis of the Symbiondinaceae in the presence of ammonia, resulting in bleaching. They also highlighted that many of these virulence factors: adhesin, toxin, and superoxide dismutase, are produced only at elevated summer seawater temperatures—again showing the role climate change plays in coral health and bringing us back full circle to a multi-faceted cause of coral bleaching.

12.1.2 The Case of *Vibrio coralliilyticus* and Mistaken Identity

Other bacteria, including several more vibrios, appear to also play a role in bleaching (to some degree), and this has been evidenced via a suite of controlled experimental laboratory studies. For example, *Vibrio coralliilyticus*, which under 'normal' conditions may actually act as a commensal, can become pathogenic when the host is stressed and certain environmental conditions are met (Gibbin et al. 2019). Although the mode of pathogenicity for this species has been explored to some length, we are only starting to understand how some *V. coralliilyticus* strains initiate a bleaching response. For example, the zinc-metalloprotease, VcpA, produced by this bacterium can inactivate photosystem II in the

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endosymbiotic algae (family Symbiodiniaceae) (Sussman et al. 2009). Interestingly, *V. coralliilyticus,* while being attributed to bleaching, has also been credited as being a major player or in some cases the single pathogen associated with diseases such as white syndromes throughout the Caribbean and Indo-Pacific (see "Coral Disease" below) (see Table 1 in Ushijima et al. 2022). Interestingly, only a subset of reported infections and strains of this species are associated with signs of bleaching while tissue loss is a common sign of infection. *Porites* white patch syndrome on the other hand, which has also been accredited to being caused by another *Vibrio*, *V. tubiashii* (Séré et al. 2015), shows field signs of bleaching as well as associated tissue loss. In fact, this disease is characterized by diffuse, medium to large (50–300 mm diameter), circular to oblong tissue loss, surrounded by swollen white tissue (Séré et al. 2012). Sadly, again, there is limited further information on this disease and how the bleaching is initiated remains unexplored. It should also be noted that there is discussion over the taxonomic identification of *V. tubiashii*, in that some isolates may actually be strains of *V. coralliilyticus* (Wilson et al. 2013; Richards et al. 2015).

Somewhat surprisingly, this taxonomic 'identity crisis' is not a unique phenomenon for members of this genus. Indeed, in a comprehensive assessment of *Vibrio*-like isolates originating from bleached corals, Thompson et al. (2005) concluded that the bacteria were actually more closely related to *Photobacterium* and *Enterovibrio*. This might suggest that the plethora of next-generation sequencing studies, which have historically pointed the finger at *Vibrio* spp as causal agents of coral disease and/or bleaching episodes may have been misidentifying the potential pathogens. This is especially relevant if any project/study is reliant on 16S rRNA gene sequencing alone, i.e. this is well known for being unable to demarcate *Vibrio* spp. from one another (Sawabe et al. 2013). In the same study, the authors went on to describe two new bacterial species commonly associated with bleached coral: *P. rosenbergii* and *E. coralii* (Thompson et al. 2005). However, there have been little further developments regarding their role, other than *P. rosenbergii* being identified in marine litter, specifically plastics (Curren and Leong 2019) and *E. coralii* being more commonly associated with fish (Mladineo et al. 2016).

12.1.3 Flexible and Plastic

In recent years, attention has somewhat moved away from the concept and identification of 'individual' pathogens associated with coral health and disease and more to the role of the microbiome or pathobiome in its entirety (Sweet and Bulling 2017). Interestingly, studies show that more bleaching-resistant corals (such as *Goniastrea edwadsi*) appear to associate with a greater functional diversity of endolithic microbes than more bleaching-sensitive species such as *Porites lutea* (Cárdenas et al. 2022). The role of flexibility in this diverse coral-associated microbiota has also been noted (Voolstra and Ziegler 2020). Here, those with an ability to shift and change their microbiome (possibly acquiring new symbionts from the water column or sediment for example) are postulated to be able to adapt and change their thermotolerance in a more rapid manner. However, this 'flexibility theory' is also not straightforward. The *Pocillopora verrucosa* microbiome is often rather conserved, and the corals maintain their microbiome throughout various stress events (Pogoreutz et al. 2018). That said, this species does widely succumb to bleaching. On the other hand, *Oulastrea crispata* is a coral species that is often found in less than ideal conditions, is apparently rather resistant to bleaching and disease, and yet it too has a rather conserved microbiome (Röthig et al. 2020). At least in this case, the authors concluded that 'flexibility' in the microbiome did not appear to underpin the robustness of this broadly distributed coral (Röthig et al. 2020).

To add to the mix, work focusing on the use of probiotics in coral health has shown the potential to increase coral resistance to bleaching through microbiome stewardship (Rosado et al. 2019). Both metabolomic and genetic restructuring of the host has been accredited as a possible mechanism for how the corals are able to mitigate this heat stress. However, we still do not know how much is driven by the coral's microbiome or the host itself (Santoro et al. 2021).

12.2 Coral Disease

12.2.1 An Ode to Pathogenic Microbes

Infectious diseases are, by nature, caused by pathogenic organisms disrupting the normal functions within a host. Pathogenic microbes have existed since the early stages of life, which have evolved to the point where every form of life has at least a few associated pathogens. This includes the complex multi-host life cycles of parasitic worms, mind-altering fungi, down to viruses that infect bacteria or even other viruses. However, relatively speaking, humans have only just begun to understand the nuances of a pathogenic lifestyle. Microorganisms were only first observed in the mid-1600s by Dutch naturalist Antoni van Leeuwenhoek with his primitive, yet groundbreaking, prototype of the light microscope (Gest 2004). By the 1700s, the advent of interventions against the deadly disease smallpox (caused by the *Variola* viruses) spread to western medicine by Lady Mary Montagu (variolation) and Edward Jenner (vaccination) (Jenner 1801; Dinc and Ulman 2007).

However, we had to wait until the 1800s for a connection to be made between diseases and pathogenic microbes. Various scientists like Louis Pasteur demonstrated that the concept of spontaneous generation (the appearance of life out of nothing) was actually contamination by unseen entities (microorganisms). However, his specific treatment protocols (later coined pasteurization) could prevent spoilage by killing these contaminants (Doetsch 1962; Steele 2000). The associations of microbes and disease were further established by the early experiments by British surgeon Joseph Lister and his use of phenol compounds to disinfect surgical equipment, which significantly reduced mortalities after operations (Lister 1867). During this time, Robert Koch developed his experimental methodologies that firmly established the germ theory of disease, which postulated that pathogenic microorganisms were the cause of infectious disease (Ullmann 2007). Since then, the field of human medicine and disease has advanced with the advent of new discoveries and technologies. That said, although these technological advances have propelled disease studies forward, they have also revealed new dimensions to disease that Koch and his contemporaries would have never imagined. For example, studies on mammalian host microbiomes have begun to blur the lines between communicable (transmissible infections typically caused by pathogenic microbes) and noncommunicable diseases (non-transmissible conditions attributed to genetics, the environment, or lifestyle), where the state of the host microbiome may contribute to obesity, gastro-intestinal conditions, and cardiovascular disease (Finlay et al. 2020). Similarly, some studies suggest that for certain infectious diseases it might be more effective to manage the host microbiome than direct treatment of the disease agent (Kirchhelle and Roberts 2022). These examples demonstrate that disease is far beyond just the “if exposed to a pathogen, then get sick” mentality, but instead a complex, yet fascinating, network of variables that contribute to the disease process.

12.2.2 A Brief History of Coral Disease

Compared to human disease, the field of coral disease is relatively new and has not had the time to develop as far as related fields. The first coral disease was only formally described in the late 1970s and published in the early 1980s (Antonius 1981). This “band disease” was later named black band disease (BBD). Although this was the first coral disease to be described, it was likely not the first incidence of coral disease simply because disease itself was not routinely recognized as a trait to be noted or recorded before this date. Indeed, it took a series of disease outbreaks throughout the 1980s and early 2000s that would shine a light on the importance of disease to this ecosystem and this was the time for white syndromes “to shine”. The term “white syndrome” is used to describe tissue loss on a coral with unknown etiology named from the exposed white skeleton from the disease lesions, akin to pneumonia in people that could be caused by a number of pathogens. Thus, many of these individual diseases had been labeled with a similar naming scheme, e.g., white band type I and type II or white plague type I, type II, or type III as well as the slightly more distinctive white pox (Dustan 1977; Ritchie and Smith 1998; Richardson et al. 1998, 2001; Patterson et al. 2002; Denner et al. 2003). Unfortunately, only a fraction of these white syndromes have had their etiological agents identified (Richardson et al. 1998; Patterson et al. 2002).

12.2.3 Simple and Complex

Due to their lack of physical complexity, corals are often mistaken as “simple” organisms, yet most are a complex symbiosis between the colonial animal, photosynthetic endosymbionts, and a rich microbiome. However, disease itself is also a complex process beyond just simply exposure to a pathogen and the manifestation of disease, so investigating coral diseases in general can be a difficult task. A major principle of pathogenesis is the concept of the disease triangle (Stevens 1960). This concept illustrates that disease is an interaction between various host-, environmental-, and pathogen-associated factors (Fig. 12.1). The severity of various coral diseases correlates with specific environmental conditions like anthropogenic stressors such as sewage pollution (Kaczmarsky et al. 2005; Redding et al. 2013), while correlations have been found with plastic pollution and disease prevalence (Lamb et al. 2018). Similarly, the devastating effects of anthropogenic climate change, resulting in increased ocean temperatures, are driving the incidence of various coral diseases (Bruno et al. 2007; Ruiz-Moreno et al. 2012; Maynard et al. 2015). In contrast, multiple host factors for any disease can greatly influence disease susceptibility. While there are no reported genetic markers demonstrated to be directly linked to susceptibility to a known individual coral pathogen, coral nurseries with their tracked coral genotypes suggest a host genetic factor to disease resistance (Brown et al. 2022). Related to this, the unique microbiome associated with certain coral genotypes could be indirectly driving disease resistance (Rosales et al. 2019). These microbiome-pathogen interactions may be an important driver for disease resistance and have been the focus of using the beneficial microbes on corals to fight disease (Rosado et al. 2019; Ushijima et al. 2023).

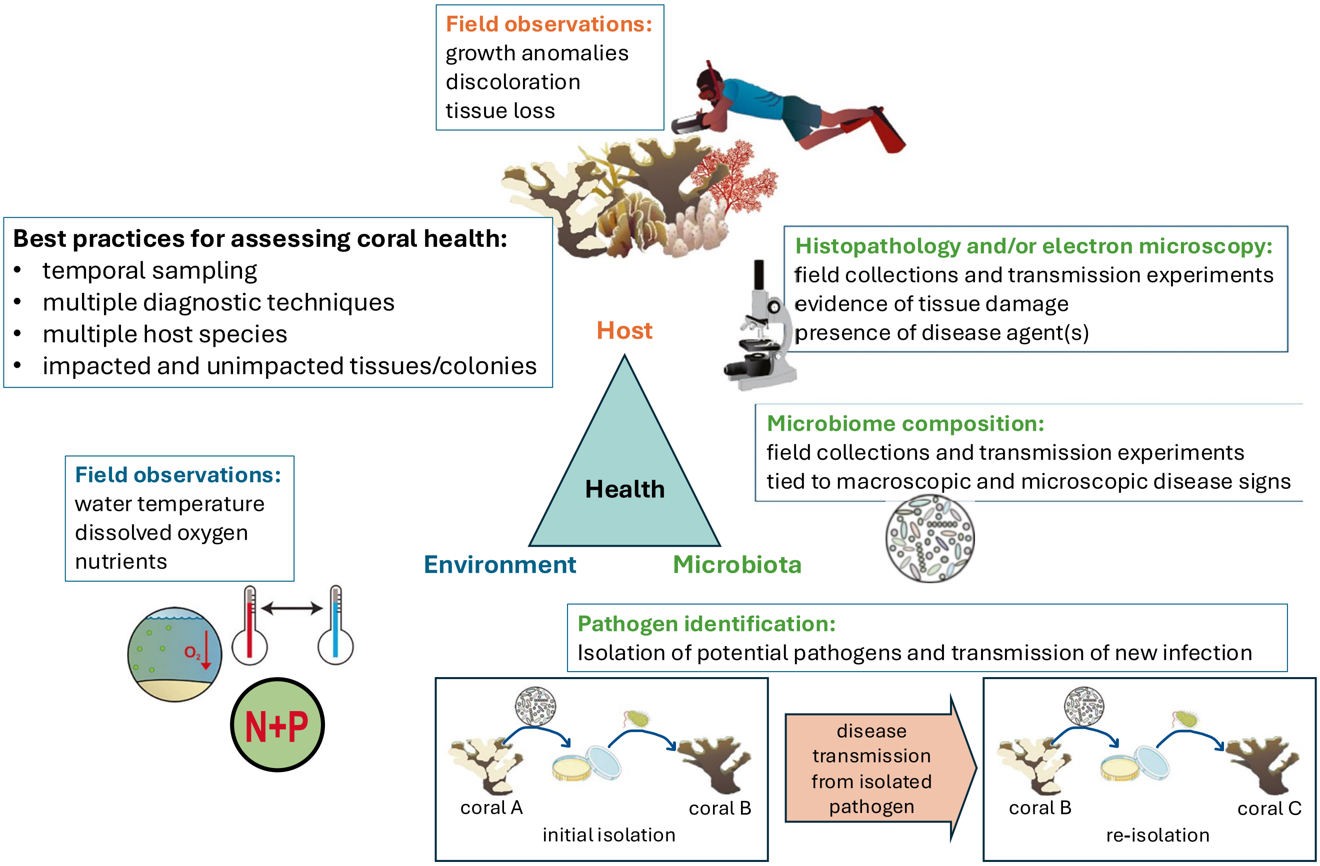

Fig. 12.1 Best practices for assessing coral health and disease. Holobiont health lies at the intersection of host susceptibility to disease, environmental stressors, and microbiome composition. Thus, best practices for the identification of causal factors should include sampling at multiple time points and the use of multiple diagnostic techniques to tie field observations of the host and environment to patterns in microbiome composition. Sampling in the field and in the lab during experimental transmission experiments would ideally include multiple host species and multiple individuals. Sampling should also include both impacted and unimpacted tissue and colonies for comparison of the presumably normal microbiota and a potentially dysbiotic microbiome. Figure includes graphics courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/)

12.2.4 Show Us a Sign

A challenging issue with coral diseases, and many other non-human infections, is the reliance on objective, observable disease *signs* that are diagnostic (Sweet et al. 2021). Unlike physicians, coral researchers do not have the luxury of patients being able to describe their subjective symptoms, such as fatigue, pain, or itching. This is further complicated by the limited range of gross disease signs that a coral can display during an infection. For all coral diseases, the disease "signs" will generally display as growth anomalies, discoloration, or tissue loss (Work and Aeby 2006). Growth anomalies are the least understood in terms of coral disease and, analogous to growths in human medicine, are hypothesized to be caused by environmental mutagens or infections from potential viral pathogens though these have yet to be experimentally confirmed (Domart-Coulon et al. 2006; Aeby et al. 2011; Ricci et al. 2022). Regardless, they typically result in the proliferation of coral tissue and skeleton that is often devoid of endosymbionts and can drain the total energy resources of a colony. Discoloration is the localized production of pigmented compounds or loss of pigmentation due to infection. This is clearly illustrated with corals infected by the digenetic trematode *Podocotyloides stenometra*, resulting in *Porites* trematodiasis (colloquially termed "coral zits") that manifests in bright pink lesions (Aeby 1998; Martin et al. 2018). In contrast, discoloration could manifest as localized bleaching caused by infectious agents. Please refer to "Bacteria and Bleaching" above, but to reiterate, some bacterial infections, like those of *V. shilonii* (now *V. mediterranei* and formally *V. shiloi*), have been reported to cause bleaching in the coral *O. patagonica* (Kushmaro et al. 2001). While difficult to demonstrate causality, there have also been various Herpes-like viral agents associated with bleaching (Correa et al. 2016). One final sign of coral disease, is of course tissue loss (commonly referred to as *white syndromes*—i.e., tissue loss lesions of unknown etiology). These diseases result in the exposure of the coral skeleton from the destruction of the coral tissue. This is the most common disease presentation for the "known" coral pathogens (Bourne et al. 2009, 2015; Vega Thurber et al. 2020), many of which may appear generally similar. However, the specific details of these lesions can manifest as different patterns with levels of inter- and intra-specific variation (Work and Aeby 2006; Bourne et al. 2015). In all, it is because of these limited number of lesion presentations that **coral diseases typically cannot be diagnosed in the field based on the observed "signs" alone**.

12.2.5 Cause or Consequence

For over a century successful disease studies have been based on the four postulates of disease causation—established by Koch and his protégés (*Koch's Postulates*) (Koch 1876; Evans 1976). These postulates are generally accepted as some form of the following:

1) *The pathogen is found in all diseased hosts, but not healthy hosts.*
2) *The pathogen can be cultured and purified from diseased hosts.*
3) *The purified pathogen can recreate disease signs in exposed naïve hosts.*
4) *The pathogen can be re-isolated from infected hosts.*

While Koch's postulates are useful guidelines for the development of etiological investigations, it must be remembered they were developed in the 1800s before many advances in disease research or technologies (Evans 1976; Falkow 1988). Because of this, several misconceptions have been perpetuated stemming from these postulates, especially for complex systems like corals. One major misconception is the "one pathogen – one disease" concept, which is an assumption made by Koch's postulates that a single etiological agent is always responsible for an infection. It is true that there have been some 'individual' pathogens identified as causal agents of specific disease signs like *Serratia marcescens* causing acroporid serratiosis (formally white pox disease) (Patterson et al. 2002) or *V. coralliilyticus* and white syndromes/vibriosis (Ben-Haim et al. 2003; Sussman et al. 2008; Vezzulli et al. 2010; Ushijima et al. 2016). However, the mere concept of "one pathogen" can have numerous caveats associated with it. A singular bacterial species may consist of numerous biovars that include pathogenic and commensal strains, which, right from the start, invalidates the assumptions made by Koch's postulates. For example, when various strains of *V. coralliilyticus* were tested against *Montipora capitata*, a coral susceptible to this pathogen (Ushijima et al. 2014), only specific strains were able to induce tissue loss (Ushijima et al. 2022). In fact, Ushijima et al. (2022) found one *V. coralliilyticus* strain, H1, that was originally isolated from healthy *M. capitata* and was completely avirulent to this coral.

In contrast, some coral diseases are polymicrobial infections that require a suite of microbes present to initiate disease (Sweet and Bulling 2017). An excellent example of a polymicrobial disease is the previously mentioned BBD, which is a consortium of a cyanobacterial mat with various sulfide-oxidizing and sulfate-reducing communities (Cooney et al. 2002; Sekar et al. 2006; Meyer et al. 2016a). Signatures of some of these BBD constituents have been found individually on healthy corals; for example, the cyanobacterium, *Roseofilum* sp., can be found on healthy corals but is an essential component of BBD as well as responsible for the distinctive *black band* on diseased corals (Meyer et al. 2016a). Interestingly, the polymicrobial infections of BBD appear to consist of taxonomically different constituents depending on geographical location, which can even have cyanobacterial species belonging to different families (Rützler and Santavy 1983; Sussman et al. 2006; Rasoulouniriana et al. 2009; Casamatta et al. 2012; Aeby et al. 2015; Meyer et al. 2017). This aspect of BBD strongly suggests that the functional roles play a much larger part for BBD than taxonomic classification.

Similar to polymicrobial infections, there can be cases of secondary or co-infections that further tangle the 'one pathogen, one disease' concept. *Secondary infections* are scenarios where a primary (frank) pathogen initiates disease in a host, which allows for a secondary pathogen (which does not normally cause disease), to infect (Sweet and Brown 2016a, b). While secondary infections are well-described in human medicine (e.g., bacterial pneumonia following a COVID-19 infection), it is less reported in the coral realm. One potential example is with the coral pathogen *Thalassomonas loyana* that causes tissue loss with the coral *Favia favus* (Thompson et al. 2006), but only if this bacterium is combined with a "filterable factor" that can pass through 0.2 μm-pore filters (i.e., a particle smaller than most bacteria) (Barash et al. 2005). While this fact does not technically designate *T. loyana* as a secondary pathogen, if this filterable factor were, hypothetically, an infectious viral particle causing a primary infection that allows for *T. loyana* to now infect, then that would designate this bacterium as a true secondary pathogen. Contrary to secondary infections, a *co-infection* occurs when infection by a primary pathogen allows for another pathogen to better infect a host, but the primary infection is not essential for the co-infection. However, these co-infections can potentially exacerbate the pre-existing disease and mask the discovery of a primary pathogen. This is demonstrated by the bacterium *Pseudoalteromonas piratica*, which can directly infect *M. capitata*, albeit at relatively low rates, causing acute (rapid) tissue loss (acute *Montipora* white syndrome) (Beurmann et al. 2017). However, Beurmann et al. (2017) described how if the corals had a pre-existing infection with a chronic tissue loss disease (chronic *Montipora* white syndrome) (Ushijima et al. 2012), then

P. piratica infected at comparatively higher rates and accelerated the progression of tissue loss. Interestingly, Pacific corals with pre-existing chronic *Montipora* white syndrome lesions did not appear more susceptible to the previously mentioned pathogen *V. coralliilyticus* (Beurmann et al. 2017), which is hypothesized to be causing co-infections in the Caribbean with corals afflicted with stony coral tissue loss disease (SCTLD) (Ushijima et al. 2020). Unfortunately for SCTLD, the *V. coralliilyticus* co-infections are miniscule compared to the damage this disease has done, which has devastated reefs throughout Florida and throughout the greater Caribbean (Papke et al. 2024).

Another major issue for coral disease, or any disease for that matter, is the inability to easily culture every pathogen. In general, the *Great Plate Anomaly* suggests that for many environmental samples a majority of the microbial cells (which could range from 90–99.9%) are not culturable on a growth medium (Staley and Konopka 1985). In many occasions this is due to our inability to recreate the complex nutritional requirements, physical environment, or microbial interactions that are essential for growth (Joint et al. 2010; Stewart 2012; Lagier et al. 2015; Overmann et al. 2017). Further, some pathogens may enter a VBNC state (mentioned above) and are not culturable but still infectious (Colwell et al. 1985), which has been shown with some coral pathogens (Vattakaven et al. 2006). However, this is all under the assumption that the pathogen can survive outside of a host as various obligate intracellular pathogens can only be grown within an infected specimen. While there have been great strides in culturing microbes using, for example, *in situ* culturing devices (Berdy et al. 2017; Modolon et al. 2023), some pathogens remain unable to grow in axenic cultures. This is epitomized by all viral pathogens, which can only replicate their genetic material utilizing host machinery and cannot be axenically cultured. This imposes severe limitations to the study of viral pathogens, which require more intensive methods for study like RNA sequencing and electron microscopy (Thurber et al. 2008; Correa et al. 2016; Work et al. 2021). However, as molecular biology technologies become more advanced and a greater diversity of expertise becomes associated with coral disease research, this will change and improve how coral diseases are investigated.

12.2.6 In with the New

While the application of concepts like Koch's postulates have led to the identification of some of the world's most devastating pathogens, they were still developed before the advent of the various sequencing technologies or even the discovery of DNA as the heritable material of genetic information (Evans 1976; Falkow 1988). Therefore, there have been various endeavors to update Koch's postulates to coincide with current knowledge and technologies. For example, in Falkow (1988) a new set of *molecular postulates* were proposed to highlight and utilize the concepts of genes encoding *virulence factors*, cellular structures/molecules that contribute to overall virulence and pathogenicity. This application accounted for more virulent strains of pathogens versus their non-pathogenic counterparts as well as helped to demarcate the roles of opportunistic pathogens. However, these postulates still mostly relied on the ability to culture and now genetically modify a pathogen, which could be problematic for environmental systems and microbes. Another undertaking was described in Fredricks and Relman (1996), which lessened the dependence on axenic cultures of pathogens by utilizing nucleic acid (DNA/RNA) amplification and sequencing methodologies as well as advancements in molecular biology and microscopy. These amendments to past concepts were steps forward in developing more adaptable approaches to studying diseases, which helped pave the way for more field-specific paths focused on environmental pathogens. For example, Sweet and Bythell (2017) proposed approaches to account for these issues as well as develop guidelines for coral disease studies specifically. They propose that researchers:

(1) Demonstrate consistent enrichment of the proposed causal agent (or sequences related to it) in all cases of the disease in question (spanning different locations and where possible, different host species).
(2) Characterize the disease in question using a suite of methods, including field observations (e.g. via tagged and monitored colonies, assessing lesion progression); on a cellular level (e.g. immuno-histopathology or electron microscopy) and comprehensive assessment of the microbiome in healthy, apparently healthy, and diseased colonies (e.g. metagenomics or transcriptomics). A stronger case can be made where the disease pathogenesis can be both temporally and spatially (microscopically) correlated with activity of the suspected pathogen.
(3) Isolate the suspected agent/agents in culture and expose multiple, independent healthy host samples to these isolates with adequate controls (i.e. non-suspected members of the microbiome). As we recognize a lack of adequate cell culture models precluding the culture of coral-associated viruses (as discussed above), this step may be limited at present to applications of filterable fractions (e.g. <0.22 μm) to distinguish bacterial cells from viral particles infection, for example (Evans et al. 2022).
(4) Use whole-microbiome analyses to demonstrate that exposure (3) does not upregulate any other members of the microbiome in advance of the characteristic disease signs (2).
(5) Where (4) cannot be met, isolate, and test any enriched members of the microbiome as control inocula.

(6) Assess the probabilistic risk of producing the disease signs (2) upon exposure (3 or 5), under a range of environmental conditions.
(7) Demonstrate that the up-regulation of the suspected agent (or agents) in experimental trials is of a similar magnitude to those observed in field samples of the disease.

However, even ‘completion’ of this more detailed/modern version of the disease postulates, does not necessarily mean the pathogenic agents of certain diseases would be revealed. For example, does a pathogen need to be present throughout all stages of the infection? And even if it is present, it almost certainly does not need to be present in high abundances. The former could be explored by ensuring samples are collected in a time series, which remains rarely undertaken but the latter issue still presents problems with no obvious solutions to date.

12.3 Dysbiosis

12.3.1 Imbalance of the Microbiome

The concept of dysbiosis has gained popularity as the investigation of animal microbiomes has accelerated, but its utility in investigations of microbial ecology has been questioned due to the ambiguity of the concept (Olesen and Alm 2016). Although the term dysbiosis was first applied to the microbiome more than a century ago (Scheunert 1920), we still lack a clear consensus on its definition and its application in host-associated microbial ecology (Hooks and O’Malley 2017). In broad strokes, dysbiosis is an alteration of the microbiome composition correlated with a negative health state in the host. However, in most studies of dysbiosis, the mechanisms behind these changes in composition are not investigated and the use of single temporal snapshots of microbial communities cannot distinguish if the change in microbiome composition is a cause or consequence of disease (see above). Comparisons of the microbiome composition in apparently healthy tissue and tissue with disease signs is common (Closek et al. 2014; Meyer et al. 2019; Rosales et al. 2020; Becker et al. 2021; Schul et al. 2023), but can be limited by our understanding of what the healthy microbiome should look like, as these comparisons are relative to the benchmark chosen. In addition, what “healthy” looks like will vary with season, location, and host species (Roder et al. 2015; Dunphy et al. 2019; Ziegler et al. 2019; Dubé et al. 2021; Voolstra et al. 2024).

Regardless of our definition, most agree that dysbiosis can manifest as an imbalance of the microbiome or as a change in the composition of the microbiome, especially when changes are characterized by the invasion of putative pathogens (singular or plural). The term “imbalance” in this context, implies that the microbial community members have not changed, but rather that some taxa have changed in abundance, reflecting a critical alteration of ecological relationships within the community. We may recognize this imbalance by examining changes in the evenness of community diversity or by determining taxa that are differentially abundant yet present in both healthy and diseased tissues (Sweet et al. 2019; Vega Thurber et al. 2020; Rosales et al. 2023). Here, we use the term “diseased” to include any host tissue with signs of damage, regardless of the cause, as is typical in human health studies. Thus, disease and dysbiosis in coral may be the result of environmental stress and present as bleaching or tissue loss, as detailed above. Importantly, it should be noted that this could occur with or without an infectious causative agent being present (Sweet and Brown 2016a, b).

12.3.2 Constrained Chaos

In corals, dysbiosis of the bacterial community has been most commonly described in coral disease, tissue loss, and bleaching (Meyer et al. 2016b, 2019; Quintanilla et al. 2018; Keller-Costa et al. 2021; Silva-Lima et al. 2021; MacKnight et al. 2021; Clark et al. 2021; Huntley et al. 2022). However, cases of dysbiosis have also been reported in coral-predator studies (Bettarel et al. 2018; Ezzat et al. 2020; Clements et al. 2020), and studies of the impact of fish feces on coral health (Ezzat et al. 2019, 2021). This latter finding could arguably be linked with changes in nutrient load around the coral, however, nutrient stress alone appears to have relatively minimal impact on the host's microbiome in some coral species (Maher et al. 2019, 2020). Similarly, community shifts have also been observed in conjunction with human-sourced sewage pollution which increased nitrogen content in the surrounding water as well as fecal indicators in the coral microbiome (Leite et al. 2018). Thus, the introduction of both fecal bacteria and extra nutrients may be needed to induce dysbiosis.

Regardless of whether the cause of dysbiosis is correlated with external environmental factors or by compromised host immunity, the resulting microbial community is hypothesized to assemble stochastically after disturbance, rather than deterministically such that each dysbiotic community is unique (Zaneveld et al. 2017). This is consistent with the idea that a compromised host would be less capable of “curating” its microbiome, thus allowing colonization or increased growth of opportunistic pathogens and saprophytes. In other cases, community diversity does not increase with dysbiosis or disease; instead, certain taxonomic groups that are already present in the coral microbiome and that appear to favor disturbance may be enriched. We could describe this phenomenon as “constrained chaos” or compositional homogeneity, as observed in the polymicrobial ‘grey-patch disease’ correlated with cyanobacterial mats

(Sweet et al. 2019). Selection of sample types may also critically influence observed patterns in community diversity. For example, a meta-analysis of SCTLD studies revealed that alpha-diversity was highest in visually unaffected tissue on diseased colonies, while alpha-diversity was similar between disease lesions and visually unaffected tissue on healthy colonies (Rosales et al. 2023).

12.3.3 The Rise of Anaerobes

Disturbance of the coral microbiome is often associated with an increase in anaerobic bacteria such as *Arcobacter* (Phylum Campylobacterota), *Desulfovibrio* (Phylum Desulfobacterota), and Peptostreptococcales [Phylum Bacillota]. Sulfur-oxidizing Campylobacterota and sulfur-reducing Desulfobacterota are ubiquitous in low-oxygen marine settings, and it is not unexpected to see their increase during decomposition associated with coral tissue loss. In contrast, among anaerobes associated with corals, Peptostreptococcales [formerly Clostridiales] may be of particular interest for their unique roles in dysbiosis and disease (Sweet et al. 2011). For example, it is well documented that after disturbance with antibiotic treatments, *Clostridioides difficile* [formerly *Clostridium difficile*] can establish destructive infections in the human digestive tract, particularly in immunocompromised patients. *C. difficile* is considered a member of the normal gut microflora but can only infect intestinal cells after dysbiosis disrupts the protective barrier of commensal bacteria lining the gut. Host damage ensues when sufficient numbers of *C. difficile* cells produce tissue-damaging toxins.

The production of toxins is also well known in soil-dwelling Bacillota, especially those that cause human diseases such as *Clostridium tetani*, *Clostridium botulinum*, and *Bacillus anthracis* which result in tetanus, botulism, and anthrax, respectively. Many Bacillota are ubiquitous in terrestrial soils, but in marine sediments they appear to be more abundant in coastal sediments than in the deep sea (Zinger et al. 2011). This suggests that Bacillota in marine sediments may ultimately be sourced from terrestrial runoff or wastewater outflow in coastal areas. Coastal reef sediments may therefore be a reservoir of anaerobes and potential pathogens, especially spore-forming Gram-positive bacteria like Bacillota.

In the coral microbiome, we have documented an increase of Peptostreptococcales and Clostridiales after disturbance with multi-day antibiotic treatments (Sweet et al. 2011), after exposure to fish farm effluent (Garren et al. 2009), in response to short-term hypoxia (Howard et al. 2023), and in lesions of SCTLD (Meyer et al. 2019; Clark et al. 2021; Rosales et al. 2023). Similarly, proteins from obligate anaerobes, including Clostridiales, were more predominant in disease lesions of white plague and black band disease compared to healthy corals (Garcia et al. 2016). While the growth of strict anaerobes like Peptostreptococcales and Clostridiales would not be favored during the day when Symbiodiniaceae are actively photosynthesizing, diel cycling between high and low oxygen conditions in the surface mucus layer (Shashar et al. 1993; Gardella and Edmunds 1999) may allow the persistence of anaerobes at low abundance in healthy corals. In addition, there is recent evidence that these strict anaerobes can detoxify and consume oxygen (Morvan et al. 2021). Just like *C. difficile* in the normal flora of the human digestive tract, Peptostreptococcales in the coral microbiome may be constrained by other commensal microbiota in addition to oxidative stress. When ecological disturbances result in dysbiosis and low oxygen conditions at the microscale, Peptostreptococcales can flourish. To date, Peptostreptococcales have not been established as disease agents in coral, but the potential for toxin production in this group and their documented increase during dysbiosis of the coral microbiome certainly warrants further investigation.

12.3.4 Change Is as Good as a Rest

So, we define dysbiosis as “a change in microbiome composition, presumably with a concomitant change in function, in a diseased host”. But is dysbiosis always going to be bad? Likewise, can we assume that a static or stable microbiome is always good? A shift in microbiome composition and function in response to stress and the stochastic assembly after disturbance may actually be an adaptive feature. For example, corals are known to shuffle the composition of the Symbiodiniaceae community after bleaching, selecting strains that are more heat resistant (Baker et al. 2004; Berkelmans and van Oppen 2006; Jones et al. 2008). Likewise, dysbiosis may present the opportunity to shuffle bacterial and archaeal members of the coral microbiome, resulting in the selection of microbes that may mitigate stress (Reshef et al. 2006). This is the motivation behind understanding and using Beneficial Microbes for Corals (BMCs) (Peixoto et al. 2017), as discussed elsewhere in this book. Resilience of the coral holobiont may depend in part on the ability to alter the composition of the microbiome under varying environmental conditions that therefore results in an *adaptive dysbiosis*. The functional redundancy in microbiomes would allow for the selection of the best-suited strains to perform necessary functions in the coral holobiont under prevailing conditions, as posited in the Coral Probiotic Hypothesis (Reshef et al. 2006). Therefore, more useful metrics for holobiont health may include investigating both functional and taxonomic stability of microbial communities. Predicting the ultimate outcome of the complex interactions within the coral holobiont will rely on a more thorough characterization of the functional capacity of coral-associated microbes. Future studies should therefore aim to include integration of metagenomic and metatranscriptomic approaches (Daniels et al. 2015; Traylor-Knowles et al. 2022; Mohamed et al. 2023), culture-based physiological measurements (Sweet et al. 2021), and visualization of micron-scale interactions through advanced microscopy techniques (Papke et al. 2024). Temporal sampling of the microbiome during experimental coral disease transmission will also be critical to unraveling the dynamics of the pathobiome. This is particularly important because primary pathogens may only initiate disease or dysbiosis and be replaced by opportunists and copiotrophs during later stages of infection (Voolstra et al. 2024; Vega Thurber et al. 2020). Also, novel analytical methods integrating artificial intelligence approaches, such as Machine Learning, may help to separate the wheat from the chaff among the sea of microbes (Staab et al. 2024).

12.3.5 Resilience Begins at the Microbial Scale

In conclusion, the vast microbial world remains mostly underexplored and we are just starting to unravel many of its mysteries especially regarding the roles and functions of coral-associated bacteria. As coral reefs decline globally, we may start to witness major shifts in microbial species abundance including the extinction of species and especially key strains (Weinbauer and Rassoulzadegan 2007; Cavicchioli et al. 2019). Microbiome shifts due to climate change and loss of coral hosts may include the loss of both beneficial and harmful bacteria or alteration of the roles that these microbes play under varying environmental conditions. In addition, the emergency use of antibiotics and probiotics in the field as an intervention to reduce "bad" microbes specifically may have unforeseen effects on beneficial members as well (Garcias-Bonet et al. 2023). It is clear that coral holobionts are the canaries in the coal mine and preservation of the ecosystem is going to be vital to ensure a one health aspect is maintained (Sweet et al. 2021), as corals, coral reefs, and indeed all marine and terrestrial ecosystems and organisms are connected, often through their microbiomes (Peixoto et al. 2022). The development of diagnostic tools will undoubtedly assist researchers and managers alike understand more about what a healthy microbiome comprises or when a healthy microbiome shifts to a pathobiome state, and these tools will be built into the general tool kit of a coral conservationist. Further, as more and more focus on out-plantation of *ex situ* reared coral recruits, attention needs to remain on the possibility of introducing unknown pathogens or potentially pathogenic organisms. Yet the cost of inaction is high and so only time will tell.

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13 Microbial-Based Therapies to Restore and Rehabilitate Disrupted Coral Health

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Abstract

Corals are facing diverse threats that disrupt their microbial symbioses, leading to changes in microbiome assemblage as a response to environmental stress. This shift affects coral physiology and resilience and is often signified by a dysbiotic state where beneficial microbes are replaced by pathogenic ones. At the same time, the malleability of the microbial assemblage provides an opportunity for intervention through targeted management of the microbiome to restore or rehabilitate disrupted coral health, a concept coined microbiome stewardship. Different approaches have been proposed to regulate and modulate, i.e., manage, the coral microbiome, such as the use of probiotics, prebiotics, postbiotics, microbiome transplantation, or phage therapy. Additionally, leveraging the sea anemone Aiptasia (*sensu Exaiptasia diaphana*) as a model organism may accelerate discovery of microbiome control mechanisms and beneficial bacteria, thus informing the development of intervention techniques. Recent results have validated the efficacy of microbiome-targeted interventions, showing substantial improvements in coral resilience in both laboratory settings and field trials. Such advancements hold implications beyond the realm of coral reefs for planetary health and ecosystem stability.

Keywords

Microbiome · Metaorganism · Holobiont · Stress resilience · Host-microbe interactions

13.1 Introduction

Coral restoration is increasingly undertaken as an active intervention to counter the global loss of reef cover with emphasis on increasing the resilience and adaptive capacity of coral holobionts (Peixoto et al. 2024; Voolstra et al. 2021). Among these efforts, the concept of microbiome stewardship, i.e., the targeted management of microbiomes to increase organismal and ecosystem resilience, is emerging as a promising intervention approach beyond the realm of corals (Peixoto et al. 2022), including humans (Daliri et al. 2018), plants/agriculture (Berg et al. 2021), and other wildlife (Peixoto et al. 2022). Microbial therapies represent one type of intervention to restore or rehabilitate the coral microbiome. Such intervention can be achieved through a number of different approaches, which include the bioaugmentation of specific microbes (i.e., through the isolation and reapplication of native microbial groups that can be sensitive to environmental impact), the introduction of exogenous microbes and/or genetically modified microorganisms with presumed beneficial traits, biostimulation (e.g., through the use of specific substrates that can enrich specific microbial populations), or the manipulation of other environmental variables (e.g., pH, temperature, light, salinity) to trigger microbiome reassembly (Voolstra et al. 2021; Santos et al. 2011).

While the framework for such applications, especially bioaugmentation, has been defined for corals (Peixoto et al. 2017, 2021), and the proof of principle has been established (Rosado et al. 2019; Santoro et al. 2021), the underlying mechanisms are far from understood, incentivizing further research efforts (Santoro et al. 2025; Dörr et al. 2023; Peixoto et al. 2021; Puntin et al. 2022; Mohamed

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et al. 2023; Voolstra et al. 2024). Current gaps in our knowledge of coral host-microbe and microbe-microbe interactions include (1) the applicability of microbiome stewardship across species (e.g., through the use of universal microbial therapies vs. custom-made designs for different coral species), (2) knowledge of the duration of microbial interventions and persistence over time, (3) the molecular underpinnings of how bacteria exert their beneficial effects on host homeostasis and resilience, and (4) the process of bacterial colonization following isolate provisioning (Voolstra et al. 2024).

The natural presence of coral-associated microbes that provide beneficial functions to the coral holobiont was the foundation for the Coral Probiotic Hypothesis (Reshef et al. 2006), which spurred the development of the microbiome as a target of active intervention (Peixoto et al. 2017). Thus, at the center of probiotic interventions lies the successful screening and identification of Beneficial Microorganisms for Corals (BMCs) (Peixoto et al. 2017), i.e., putative probiotics with beneficial traits either known or hypothesized to promote coral health. The application of BMCs (e.g., probiotic inoculation) has shown promise as a therapy for corals during and after environmental stress events that typically cause dysbiosis (i.e., detrimental microbiome imbalance) (Santoro et al. 2021; Peixoto et al. 2022). In addition to probiotic inoculation, other microbial–based approaches have been proposed and/or implemented, such as the use of prebiotics, postbiotics, and bacterial adaptation (Maire and van Oppen 2022; Schul et al. 2022; Thatcher et al. 2022; Doering et al. 2023; Garcias-Bonet et al. 2023; Rosado et al. 2023). These microbial therapies take advantage of the mutualistic relationships between corals and their associated microorganisms and allow for targeted administration of selected microbes, microbial products, or microbial substrates (Garcias-Bonet et al. 2023). Additional microbial-based methods include microbiome transplantation (Doering et al. 2021) and the top-down control of bacterial populations via phage therapy (Efrony et al. 2007; Cohen et al. 2013).

This chapter introduces the state-of-the-art and future perspectives of microbial-based therapies. It also presents current approaches to screen for and select BMCs and their application to support coral restoration efforts. The chapter further discusses emerging methods, including postbiotic and prebiotic supplementation, microbiome transplantations, and phage therapy, as well as the potential effect microbiome stewardship may have on coral epigenomes. Lastly, the chapter presents the sea anemone Aiptasia as a model system to advance microbiome-based interventions as it allows to experimentally re-configure Aiptasia metaorganisms to untangle microbial interactions.

13.2 Probiotics, Prebiotics, and Postbiotics

Probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the recipient” (Hill et al. 2014). Prebiotics, on the other hand, are compounds that can foster the growth of beneficial microbiome members (Davani-Davari et al. 2019) and/or select new microbial activities or strains (e.g., nutrient input). In contrast, postbiotics include any compound released or produced by microbial cells (or their components), including dead or inactivated cells, that directly or indirectly trigger benefits to the recipient (Żółkiewicz et al. 2020). Each one of these strategies can be used independently or combined to restore the original microbiome assemblage or restructure key coral-microbiome interactions that may have been affected by a stress event or other environmental impact. Such events often trigger a dysbiotic process within the holobiont, which refers to the disruption of the symbiotic relationships between the host and its associated microbiome (Egan and Gardiner 2016). Of note, microbiomes are often already altered by ongoing anthropogenic impact, making the elucidation of original or wild microbiomes a challenge (Peixoto and Voolstra 2023), prompting research into the concept of microbiome rewilding (Mills et al. 2017).

Probiotics are typically isolated and cultured mutualistic microbes of the respective host target organisms, although they can also be sourced from other hosts/sites (Garcias-Bonet et al. 2023). In this sense, selecting native microorganisms that are commonly found in the studied ecosystem might optimize their colonization potential and minimize potential risks. Ideally, the bacterial strains constituting a BMC consortium are microbes isolated from healthy coral colonies and selected based on putative beneficial traits to the holobiont (Peixoto et al. 2017, 2021; Doering et al. 2023; Rosado et al. 2023; Raimundo et al. 2024), with risk assessment steps to ensure that the chosen microbes do not pose harm to humans or marine life (Peixoto et al. 2022). Screening for the presence of a combination of desired beneficial traits is desirable, as different bacterial members may provide complementary functional roles for the holobiont (do Carmo et al. 2011; Villela et al. 2023). By assembling a diverse and non-harmful collection of BMC traits, scientists and researchers can maximize the beneficial effect of the consortium (Box 13.1 and Fig. 13.1).

Once selected and identified, BMCs are provided to corals in concentrations similar to natural conditions to serve a dual purpose. Firstly, they help prevent pathogenic microbes from outcompeting the native commensal bacteria when corals are exposed to stress. Secondly, they preserve and possibly enhance the coral microbiome’s beneficial roles, thereby strengthening the coral’s resilience against environmental

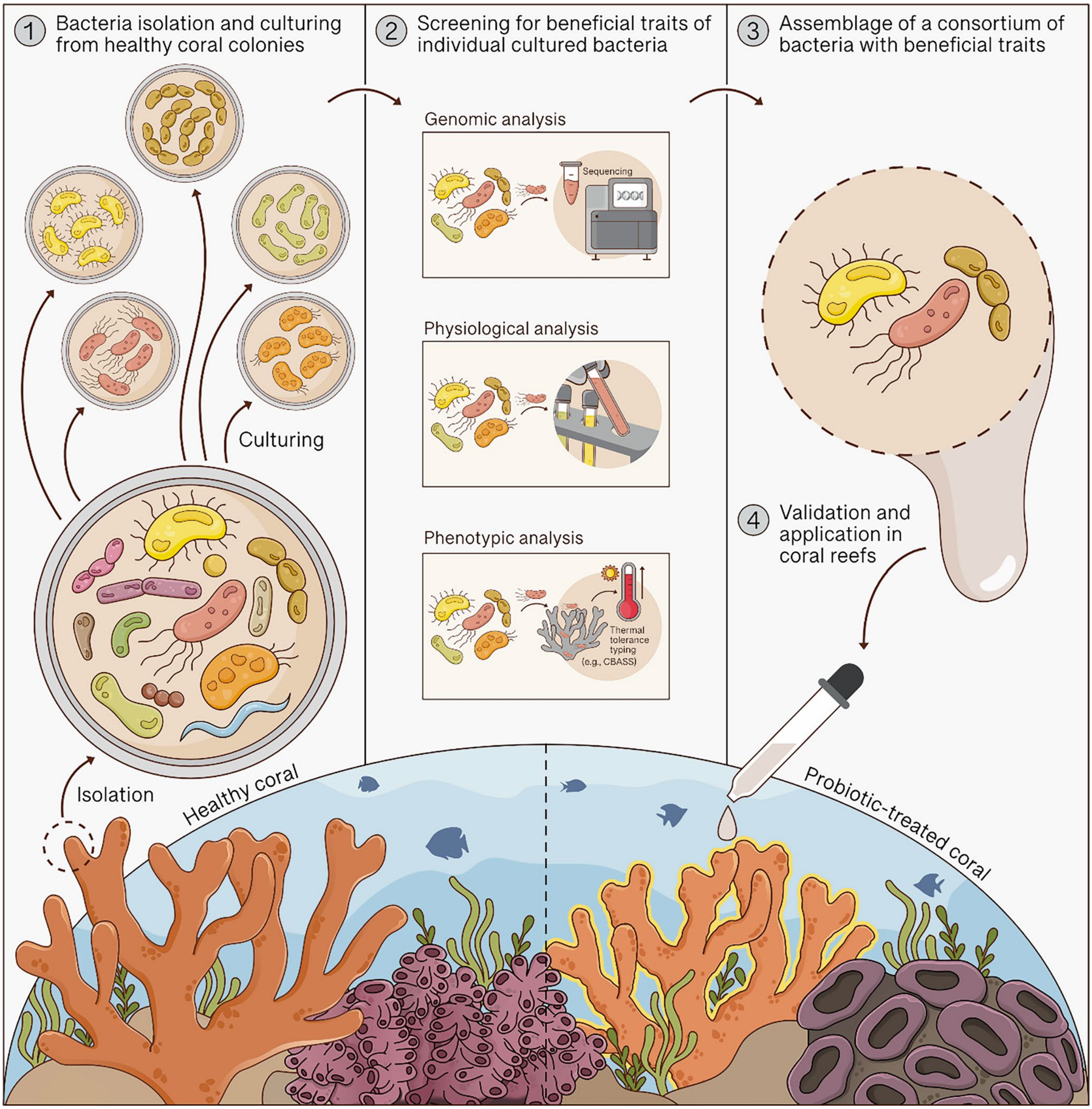
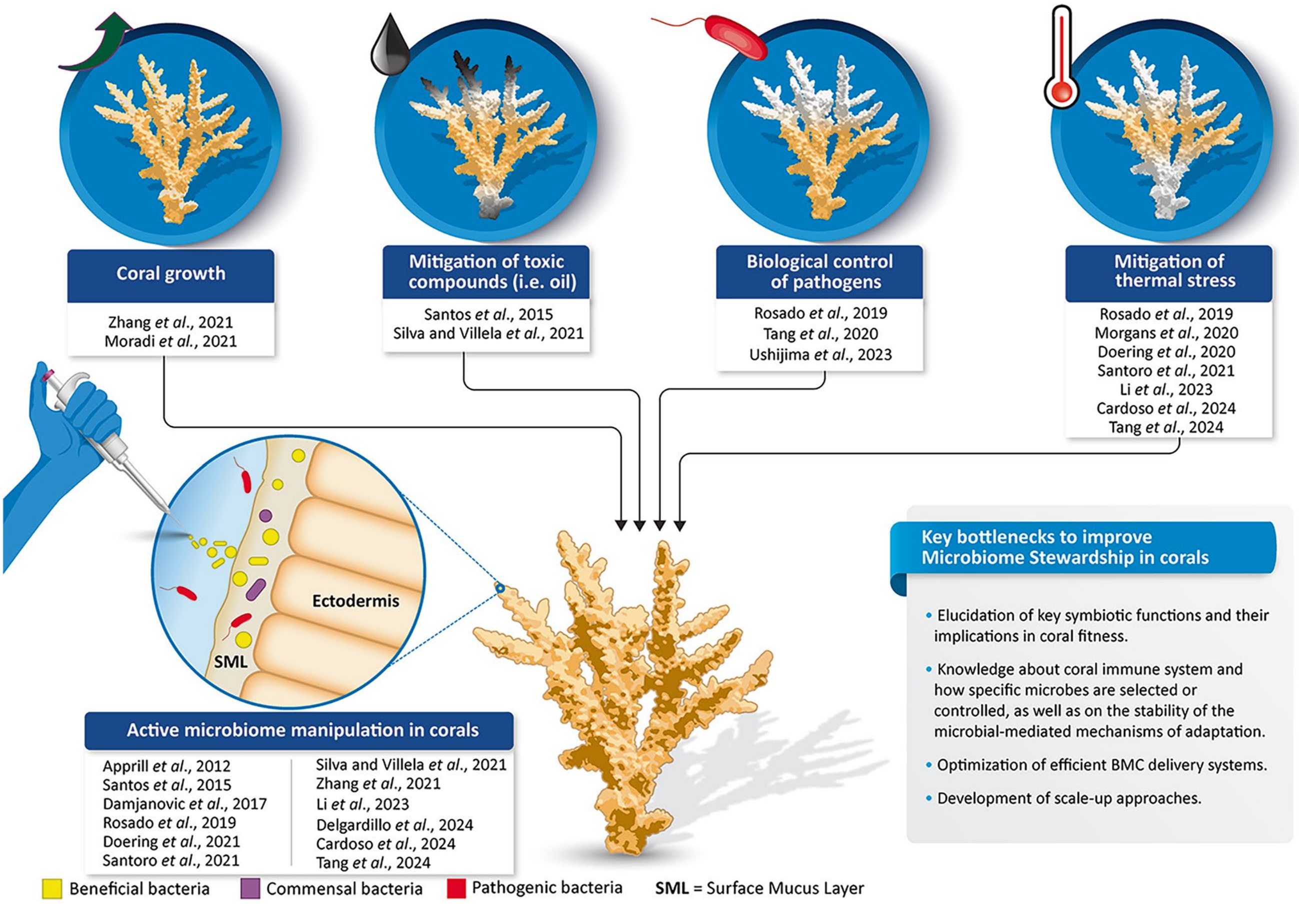


Fig. 13.1 Assembling beneficial microorganisms for coral. Depicted is the process of building a coral probiotics consortium. (*1*) A large diversity of bacteria is associated with healthy coral colonies, of which a fraction can be cultured under laboratory conditions. (*2*) Individual cultured bacteria are subjected to a series of genomic, physiological, and phenotypic screenings to identify those isolates with putative beneficial traits to host health and resilience. (*3*) A microbial consortium can be assembled using bacteria with beneficial traits. (*4*) The consortium is validated and applied to corals to treat bleached or unhealthy corals with probiotics

impacts (Peixoto et al. 2022). This stewardship of the microbiome ensures that the beneficial microbes are retained and effectively support holobiont health, fostering increased resilience during challenging conditions like heat stress and disease outbreaks - especially considering the high prevalence of coral diseases that are caused by overall dysbiotic assemblages (refer to Chap. 12). Thus, the application of probiotics, and more specifically BMCs (Peixoto et al. 2017, 2021), can mitigate an array of impacts on coral health (Fragoso Ados Santos et al. 2015; Rosado et al. 2019; Morgans et al. 2020; Tang et al. 2020; Santoro et al. 2021; Sweet et al. 2021; Zhang et al. 2021; Li et al. 2023; Moradi et al. 2023; Ushijima et al. 2023; Cardoso et al. 2024). Specifically, probiotics have been shown to improve coral resilience against bleaching and mortality caused by thermal stress (Rosado et al. 2019; Santoro et al. 2021; Li et al. 2023; Cardoso et al. 2024; Tang et al. 2024), pathogen infections (Rosado et al. 2019; Tang et al. 2020, 2024; Ushijima et al. 2023), and oil spills (Fragoso Ados Santos et al. 2015; Silva et al. 2021), as well as promote coral growth via increased coral calcification rates (Zhang et al. 2021; Moradi et al. 2023) (Fig. 13.2).

In some cases, the dysbiotic shift driven by environmental impacts is followed by lingering effects that may persist after the period of stress, such as the post-heat stress disorder (PHSD) observed in thermally-bleached corals (Santoro et al. 2021). PHSD, characterized by a host transcriptional footprint after stress, signifies a cascade of disruptions in coral health. Consequently, by restoring or rehabilitating the microbiome via probiotic application, the holobiont may recover its functions and mechanisms impaired by PHSD, thereby mitigating the effects of heat stress (Santoro et al. 2021). This approach suggests that the beneficial impact of BMCs can extend to counteracting damaging cascades triggered by various other environmental impacts. However, the precise mechanisms underlying the beneficial effect of probiotic provisioning are currently unclear. Recent evidence shows that probiotic inoculation correlates with shifts in coral metabolic and genetic processes, such as hormone production, dimethylsulfoniopropionate (DMSP) degradation, lipid maintenance, and reprogramming of cellular restructuring, repair, stress protection, and immune genes (Santoro et al. 2021; Ochsenkühn et al. 2023).

In recent years, probiotics have been effectively applied in diverse coral hosts and consist of various bacterial species such as *Actinobacterium* sp., *Pseudoalteromonas* spp., *Cobetia* sp., *Halomonas* sp., *Bacillus* sp., and *Brachybacterium* sp. (Rosado et al. 2019; Santoro et al. 2021; Li et al. 2023; Ushijima et al. 2023). Other coral genera have often been proposed as potentially beneficial, especially species of the genus *Endozoicomonas* (Doering et al. 2023), due to their consistent (Hochart et al. 2023; Delgadillo-



Key bottlenecks to improve Microbiome Stewardship in corals

- Elucidation of key symbiotic functions and their implications in coral fitness.
- Knowledge about coral immune system and how specific microbes are selected or controlled, as well as on the stability of the microbial-mediated mechanisms of adaptation.
- Optimization of efficient BMC delivery systems.
- Development of scale-up approaches.

Fig. 13.2 Overview of coral microbial therapies. Studies show the stewardship of coral microbiomes and/or coral health or growth improvements provided by the application of probiotics and microbiome transplantation (Doering et al. 2021) on corals. Bottlenecks for further improvements include elucidating microbial-mediated beneficial mechanisms and stability to enhance probiotics efficacy and scaled-up delivery

Ordoñez et al. 2022; Pogoreutz et al. 2022), endosymbiotic (Bayer et al. 2013; Neave et al. 2017a; Maire et al. 2023), and species-specific (Buitrago-Lopéz et al. 2023; Hochart et al. 2023; Neave et al. 2017a) distribution within corals (Neave et al. 2017a). However, recent evidence points towards within-genus variability indicating a rather complex relationship between corals and associated *Endozoicomonas* spp. (Neave et al. 2017b; Sweet et al. 2021; Ide et al. 2022), which may range from commensalism to parasitism and pathogenicity that need further investigation (Pogoreutz and Ziegler 2024), as also briefly mentioned in Chap. 7.

Box 13.1 How Do We Select and Assemble Beneficial Microorganisms for Corals?

BMCs should be ideally cultured from healthy corals. Culturing host-associated microbes can be challenging (Li et al. 2022; Schultz et al. 2022), but the use of *in situ* devices can increase the culturability of coral-associated microbes by up to 570%, allowing around 64% of the microbes to be cultured (Modolon et al. 2023). Additional promising improvements to enhance microbial recovery include modified culture media, such as the use of a "coral juice" to mimic the compounds found within the coral holobiont (Pogoreutz et al. 2022; Schultz et al. 2022). Once coral-associated microbes are obtained from healthy corals and taxonomically identified, the first step to assembling a putative beneficial consortium is to remove any potentially pathogenic species that are known to cause harm to any living organism. The prospective strains are then subjected to a multi-factorial screening (Peixoto et al. 2017) that can include genomic and physiological assessments (e.g., specific PCRs and biochemical assays) to identify any putative beneficial trait for corals that has been described or proposed, such as providing and/or recycling nutrients (e.g., dimethylsulfoniopropionate (DMSP), iron, and nitrogen), mitigating toxic compounds (e.g., reactive oxygen species (ROS) through ROS scavenging potential), antagonism against coral pathogens (e.g., *Vibrio coralliilyticus*), supporting larvae settlement and development (e.g., synthesizing tetrabromopyrrole), or increasing host thermotolerance (Dörr et al. 2023). The final step is to combine the beneficial roles found in the non-pathogenic candidates by assembling a microbial consortium containing (ideally) three to eight distinct strains harboring as many beneficial roles as possible (Fig. 13.1). For this, the selected bacteria should be cultured together to eliminate strains with antagonistic activity against each other. The growth curve of each of the selected BMCs can subsequently inform the growth conditions for their assemblage into a consortium and subsequent application on corals. Coral probiotics can be administered in different ways, encapsulated or not (Peixoto et al. 2021), and have until now been mostly composed of bacterial consortia, although an algal Symbiodiniaceae probiotic application and a multi-domain combination including bacteria, yeast, and filamentous fungi have also been successfully tested (Morgans et al. 2020; Silva et al. 2021). Probiotic effects must be validated against inert negative controls that do not add any confounding effects, ideally using placebos (i.e., sterile inoculation vehicles and procedures) or no-inoculation trials (Garcias-Bonet et al. 2023) in well-replicated experiments.

Although bacterial inoculation can lead to microbiome restructuring both in early life stages (Apprill et al. 2012; Damjanovic et al. 2019) and in adulthood (Fragoso Ados Santos et al. 2015; Damjanovic et al. 2019; Rosado et al. 2019; Doering et al. 2021; Santoro et al. 2021) in laboratory and field trials, inoculated bacteria are not always incorporated or enriched by the host. In some cases, they instead trigger changes in the microbiome structure and succession and/or in the host response (e.g., immune response or metabolic restructuring) that, in turn confer beneficial traits to the holobiont. This has been observed not only in corals but across different hosts (Lebeer et al. 2018; Daisley et al. 2023). An initial field trial also confirmed that probiotics do not cause measurable off-target effects to the microbiome of the surrounding water or the sediment (Delgadillo-Ordoñez et al. 2024), while resulting in microbiome restructuring and enrichment of BMCs in recipient host organisms (Delgadillo-Ordoñez et al. 2024; Ribeiro et al. 2024). Additional surveys addressing probiotic establishment and off-target effects should include other reef organisms and their health status, as well as additional sampling points and locations, in response to different inoculation regimes.

In addition to probiotics, postbiotics (in this case, dead microbial cells or microbial products) (Hill et al. 2014) can also trigger specific beneficial responses, which have been observed across several species (Wegh et al. 2019; Zhang et al. 2022; Zhao et al. 2024) and were recently suggested as an alternative therapy for corals (Garcias-Bonet et al. 2023). The holobiont responses to this therapy are specific to the composition of each postbiotic, due to different compositions of microbial cells and microbial products. A summary of some of the released compounds or structural components that trigger specific beneficial responses in different holobionts is available (Garcias-Bonet et al. 2023).

The selective enrichment of inherent probiotic strains in corals can also be enhanced through the administration of specific prebiotic compounds. Prebiotics serve as substrates that are solely used by microbes and are not bioavailable to the host, and, therefore, select specific microbes based on their capacity to metabolize these substrates. In some cases, such selection is known to shape the microbiome toward a more beneficial assembly (Hill et al. 2014). The source of nutrition also plays a pivotal role in influencing the health and microbiomes of both terrestrial and aquatic organisms (Song et al. 2014; Frame et al. 2020), with evidence of dietary changes causing microbiome restructuring in fish (Ringø et al. 2016), shrimp (Anuta et al. 2011), lobster (Meziti et al. 2012), and corals (Galand et al. 2020). In corals, alterations in nutrition sources not only affect the tissue-associated microbiome but also initiate changes in the mucus layer. This layer plays a crucial role as a first line of protection against pathogens (Shnit-Orland and Kushmaro 2009) and overall nutrient cycling in reef ecosystems (Bhagwat et al. 2024). However, some nutrient inputs, like phosphate, can also cause negative shifts in the microbiome and increase coral disease susceptibility (Klinges et al. 2022). Thus, it is essential that the appropriate prebiotics and nutrients are provided for corals, which will enrich healthy microbiomes that aid in increasing coral resilience.

Depending on the application regime and efficacy, probiotics and other microbial therapies seem to exert a more measurable effect when the holobiont is under stress (Rosado et al. 2019; Santoro et al. 2021), likely due to the different levels of stability between healthy and dysbiotic microbiomes (Zaneveld et al. 2017; Berg et al. 2020). Disrupted microbiomes change discordantly and are more prone to alteration (Zaneveld et al. 2017). In addition, the absence of a sustained microbiome restructuring effect after the end of microbial inoculation underscores a likely transient nature of these microbial changes, not only in corals but across different hosts (Santoro et al. 2021; Daisley et al. 2023). This exemplifies the role of probiotics and other microbial therapies as customized medicines (Peixoto et al. 2019), targeting short-term applications based on temperature stress forecasts, disease outbreaks, and other local impacts.

Even in the absence of measurable host health differences, microbiome changes may trigger modifications to the coral epigenome and immune responses, signifying underlying long-term resilience mechanisms that can be useful in times of stress (Barno et al. 2021). Moreover, the selection and testing of alternative putative probiotic bacterial species could generate insight into groups that can be enriched in a long-lasting way (Doering et al. 2023). For example, tissue- or Symbiodiniaceae-associated microbes, including those presenting genomic signature of symbiosis (e.g. gene loss and consequent genome reduction) (Chu et al. 2021) would be key targets to be used as probiotics that, once enriched, could be retained for longer periods of time. Microbiome management at early life stages (Apprill et al. 2012), where the coral microbiome may not be yet fully established, could also facilitate a more stable colonization by BMCs (Damjanovic et al. 2019; Voolstra et al. 2024). The future use of each of these microbial therapies will likely depend on further research and local expertise, coral reef needs, risk assessment, and other operational variables (Voolstra et al. 2021; Peixoto et al. 2021; Peixoto et al. 2019).

13.3 Microbiome Transplantation

Microbiome transplantation experiments, wherein microbial communities from donor organisms are transferred to recipient hosts, have been instrumental in elucidating the intricate relationship between microbiomes and their hosts, particularly concerning their physiological and evolutionary impacts (Greyson-Gaito et al. 2020). In clinical settings, treatments involving gut microbiome transplantation entail administering fecal material enriched with a consortium of healthy gut microorganisms, including bacteria, archaea, fungi, viruses, and yeasts. This approach has been documented to restructure disrupted (i.e., dysbiotic) microbiomes and expedite disease recovery in human recipients (Daliri et al. 2018). The primary objective of such treatments is to restore compromised gut microbiomes and alleviate microbial-driven gastrointestinal impacts. Although gut microbiome transplants have predominantly been conducted in human subjects, they have also been experimentally applied to address specific health challenges in endangered wildlife species, including dugongs, dolphins, and koalas (Eigeland 2012; Reardon 2018; Blyton et al. 2019; West et al. 2019; Linnehan et al. 2024). Rhizosphere and soil microbiome transplants have also proven effective in plant disease management, enhancing plant resistance to bacterial pathogens and boosting overall plant health (Jiang et al. 2022).

Microbiome transplantation offers the distinct advantage of transferring bacteria from selected donors that are recalcitrant to culturing in laboratory environments and potentially provides a quicker alternative to conventional microbiome therapies (Doering et al. 2021) Additionally, this method facilitates the co-transfer of symbiotic microeukaryotes, phages, and metabolites, which could be particularly advantageous for corals. In addition, host organismal traits can be screened in absence of knowledge of the underlying microbe, which allows transferring microbiome transplants of thermally superior colonies for instance (Doering et al. 2021; Voolstra et al. 2021). This includes the potential transplantation of thermally resistant Symbiodiniaceae alongside a beneficial associated microbiome. Indeed, recent studies have underscored the efficacy of this approach as a therapeutic intervention for corals and anemones, yielding promising outcomes (Doering et al. 2021; Baldassarre et al. 2022).

In applications with *Pocillopora* sp. and *Porites* sp., microbiome transplantation experiments have utilized freshly prepared tissue homogenates from heat-tolerant donors, sourced from highly variable environments. Homogenates were inoculated into conspecific heat-sensitive recipients from less variable environments (Doering et al. 2021), which enhanced heat tolerance in recipient corals, as evidenced by their improved resistance to bleaching in subsequent short-term heat stress assays. Post-inoculation, the microbiome of some recipient corals underwent significant restructuring, with specific bacterial species uniquely shared between the inoculum and the inoculated recipients, such as Rhodobacterales, *Alteromonas* sp., Alteromonadales, and Bdellovibrionales. This microbiome shift and the improved heat tolerance in sensitive recipients show the possibilities for microbiome transplantation *in situ* and the role of bacteria in conveying thermal tolerance in coral (Ziegler et al. 2017).

Although the transplantation of microbiomes from resilient corals to sensitive recipients showed promising results and could be a remarkable resource to investigate novel beneficial traits expressed in transplanted (and more heat-tolerant) microbiomes compared to native and sensitive (or less heat-tolerant) ones, its widespread adoption faces important challenges. These challenges are primarily logistical, regarding the scalability of preparation and application processes, as well as the risk of inadvertently transferring and thereby spreading pathogens (DeFilipp et al. 2019). Additionally, microbiome transplantation may transfer a diluted concentration of beneficial microbes in comparison to probiotic or postbiotic inoculations, potentially compromising the efficacy of coral health enhancement. On the upside, such approaches do not require detailed knowledge and costly equipment to isolate, screen, and handle distinct bacteria. To overcome these limitations, employing targeted approaches that utilize microbiomes with defined compositions and known concentrations of beneficial microbes may offer a more reliable and effective strategy for scaled-up in situ application (Peixoto et al. 2021).

13.4 Phage Therapy

Since their discovery in the early 1900s by Twort and d'Herelle bacteriophages became a promising and relevant agent for treating bacterial infections, evolving into what is now recognized as phage therapy. Phage therapy utilizes lytic bacteriophages (or simply "phages"), which are viruses that infect bacteria with the purpose of propagating viral progeny via cell lysis (discussed in Chap. 5) (Voolstra et al. 2021). However, due to the discovery of antibiotics in 1928 by Alexander Fleming and the rapid development and high initial efficacy of their use, phage therapy became an obsolete approach for controlling bacterial infections. More recently, however, with increased bacteria resistance to antibiotics and the need to develop alternative strategies to overcome this problem, phage therapy has again gained strength in the medical field and in environmental applications.

Phage therapy can be a powerful tool for reducing or removing pathogenic bacterial populations from the coral microbiome. Phages may be especially capable of preventing the establishment of causative agents of coral disease, because phages specifically target a single or few bacterial strains, and therefore do not kill nor affect non-target bacteria populations (as opposed to antibiotics). Furthermore, phages have high mutation rates and are able to antagonistically co-evolve with their target bacteria, leading to persistent infectivity as bacteria develop resistance to previous attacks (reviewed in Brockhurst et al. 2021). In this way, phages act as external members of the coral immune system and protect against potentially pathogenic bacteria (Silveira and Rohwer 2016).

Top-down control of bacterial populations via phage-induced bacterial lysis naturally occurs in all ecosystems. The importance of this process has been shown in coral-turf interactions, where lytic phages control the growth of pathogenic bacteria in coral-associated microbiomes (Roach et al. 2020) and within coral mucus, where lytic phages attach to mucin molecules and selectively lyse penetrating bacterial strains (Barr et al. 2013). Interestingly, bacteriophages have also been found within coral tissue and algal symbionts (Wilson et al. 2005; Howe-Kerr et al. 2023). This suggests that phages also exhibit top-down control of bacterial populations within internal coral compartments, which facilitates the opportunity for phage therapy in areas that may be inaccessible by other methods.

Phage therapy has already been successfully implemented to prevent coral tissue damage caused by bacterial pathogens *in vivo* and the spread of disease *in situ*. For example, in a pioneering phage therapy study in corals, a single inoculation of the lytic phage YB2 was able to prevent tissue loss and fragment death from consecutive infections of the coral pathogen *Vibrio coralliilyticus* in aquarium tanks (Efrony et al. 2007). A subsequent study then employed the lytic phage YC to lyse *V. coralliilyticus* in culture, thereby inactivating the pathogen when administered to coral symbionts and *Acropora millepora* juveniles (Cohen et al. 2013). Phage therapy also proved effective against the causative agent (*Thalosomonas loyaeana*) of a white plague-like disease in the coral *Favia favus*. Specifically, the lytic phage BA3 prevented tissue loss in *F. favus* when maintained in water from a diseased colony (Efrony et al. 2007; Atad et al. 2012). A follow-up study then showed that the BA3 phage was sufficient in blocking the spread of white plague-like disease in the Gulf of Aqaba, Red Sea (Atad et al. 2012). Together, the development of phages against *V. coralliilyticus* and white plague-like disease, in controlled environments and *in situ*, shows the potential for phage therapy against well-defined coral diseases.

Unfortunately, phage therapy approaches are more time-consuming and potentially more costly because phages can only be applied as a way to control one or few bacteria. In addition, specific knowledge on the bacterial strain causing disease and a lytic virus capable of infecting the bacterium effectively must both be known beforehand, as highlighted in the examples above. This, however, is rare in bacteria-driven infections in coral holobionts. Instead, the current literature indicates that coral diseases often result from altered microbiomes and groups of opportunistic pathogens (Vega Thurber et al. 2020). Thus, it is possible to target potential pathogenic drivers of coral bleaching or tissue death, but in many cases, further intervention would be necessary.

In more general terms, bacteriophages have finely controlled approaches to regulate microbial populations, playing crucial roles in microbial composition and diversity, and preventing the dominance of specific bacterial species within an environment (Naureen et al. 2020). This can be particularly useful when limiting blooms of harmful bacteria. However, the difficulties of controlling disease-causing bacteria mentioned above currently make employing phage therapy at scale in coral reefs unlikely. Thus, phages offer an interesting, albeit imperfect, opportunity to limit specific pathogenic strains within coral holobionts (Voolstra et al. 2021).

13.5 Microbe-Mediated Epigenetic Changes

One of the questions that has emerged from targeting the microbiome to improve coral resilience centers around the temporal stability of its beneficial effects on the coral holobiont. As mentioned above, microbiome changes caused by active intervention techniques may be transient. However, owing to the interrelated nature of the coral holobiont, modifications to one holobiont member group, such as the microbiome, may induce changes to others, including the coral host itself. One of the ensuing changes may be in the coral epigenome. The epigenome consists of chemical compounds that act on the DNA or RNA of an organism, controlling the gene expression, without changing the nucleotide sequence. Types of epigenetic marks include DNA methylation, histone modifications, and noncoding RNAs, although the majority of research on coral epigenomes until now has focused on DNA methylation. Coral epigenomes have been shown to be responsive to changes in the environment (Liew et al. 2018; Rodriguez-Casariego et al. 2018; Dimond and Roberts 2020; Rodríguez-Casariego et al. 2020), as is the case for coral-associated microbial communities. Likewise, both coral microbiomes and coral epigenomes can correlate with phenotypic plasticity. This implies that there exists an unexplored relationship between coral microbiomes and epigenomes.

This is not a novel concept, as microbes have been shown to affect the epigenomes of host cells in primarily humans and plants, but also gerbils and mice. Most studies in these host systems have focused on the ability of pathogenic bacteria to induce hypermethylation in promoter regions of immune-related genes, thereby diminishing the host cell's capacity to defend against infections (Ando et al. 2009). However, commensal bacteria can also suppress the overactivation of inflammatory genes via histone modifications and selective DNA methylation, particularly early in life (Takahashi et al. 2011; Bhat et al. 2019). The diminished expression of inflammatory genes then primes the organism to establish resident commensal bacteria.

Bacteria may prompt changes in coral epigenomes via several mechanisms. This could be indirect, via recognition molecules in the host cell that trigger a molecular cascade that ultimately changes the epigenetic machinery of the host cell, or direct, via bacteria-derived metabolites or proteins that translocate to the host cell, causing changes in the epigenetic marks. Coral-associated microbes provide energy and cofactors for epigenetic processes, as well as have the potential to change the physiochemical environment, which suggests an interaction between bacteria and coral epigenomes that is mediated by metabolites (Putnam 2021). Therefore, differences in nutrient availability following microbial-based therapies may lead to fluctuations in cofactors used by epigenetic machinery (Putnam 2021). Additionally, protein homologs of epigenome-modifying proteins in human-associated bacteria have been identified in coral-associated systems, indicating the possibility for protein-mediated interactions between bacteria and coral epigenomes (Barno et al. 2021).

Although this area of research is just beginning, the implications for such findings are vast and can influence the way studies employ microbiome stewardship to increase coral health and resilience. Since the microbiome is thought to be more flexible and the epigenome is considered more stable and potentially heritable (Voolstra and Ziegler 2020; Putnam 2021), exploiting a connection between these two aspects of the coral holobiont can provide evidence to support that microbiome stewardship may alter coral health in a more stable manner than commonly assumed.

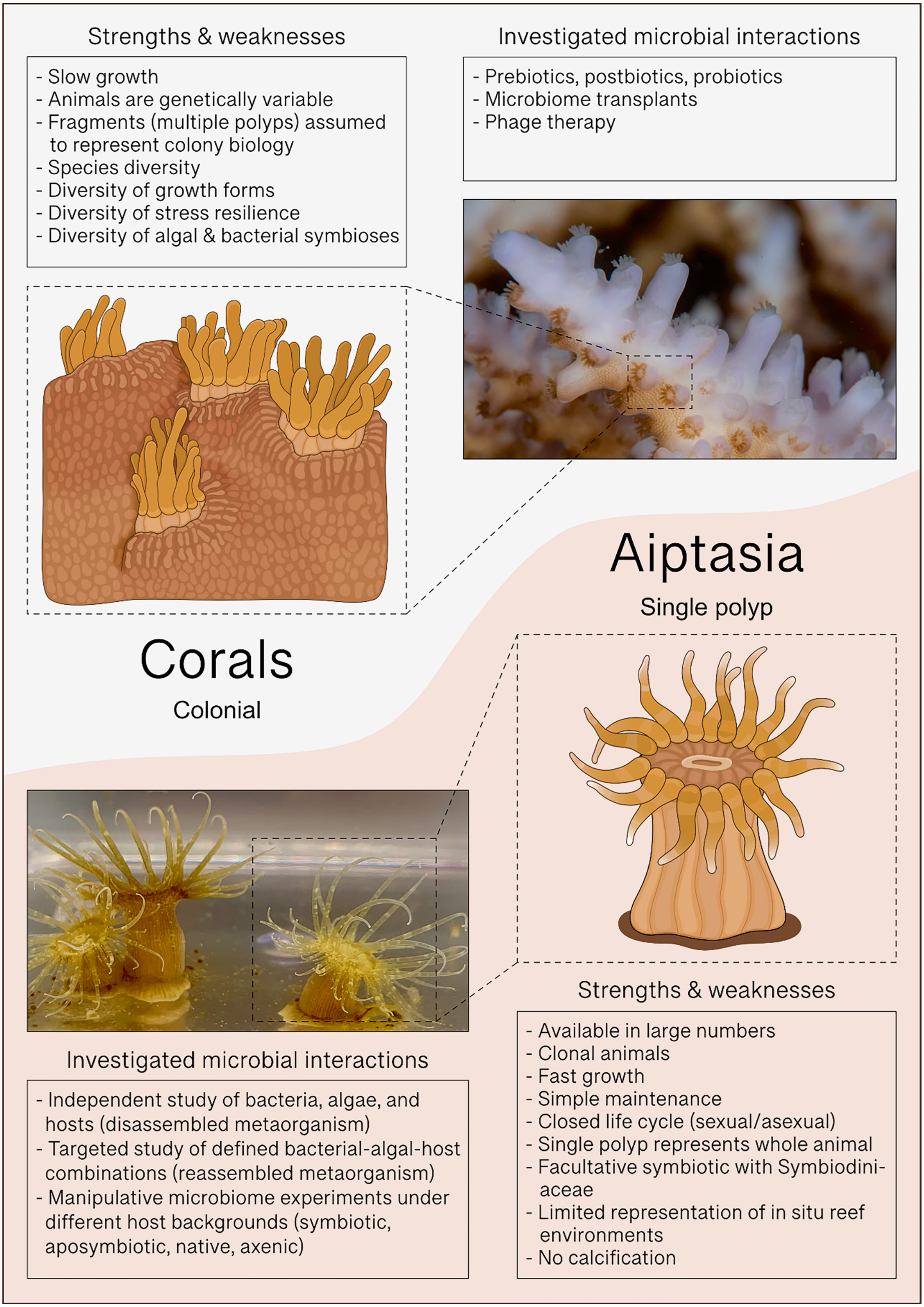
13.6 Aiptasia Model Organism-Assisted Approaches

To increase the efficacy and effectiveness of microbial-based approaches to restore and rehabilitate coral health, we must rapidly advance our understanding of coral-microbe interactions. Open questions include the applicability of the concept of microbiome stewardship across species, the duration and persistence of microbiome changes over time, the beneficial functional roles of bacteria in host homeostasis and resilience, and bacterial colonization dynamics following host inoculations (Voolstra et al. 2024). Addressing these questions in conjunction with the central need for large-scale screening and identification of BMCs (Peixoto et al. 2017) can benefit from the development of standardized approaches using model organisms that can be adopted to real-world applications (Voolstra et al. 2025). Cnidarian model organisms such as the small sea anemone Aiptasia (*sensu Exaiptasia diaphana*) (Baumgarten et al. 2015; Costa et al. 2021) are invaluable to accompany insights from field-based coral studies. Besides avoiding excess sacrifice of coral biomass, the Aiptasia model organism (1) is comparably small, fast-growing, and easy to maintain under laboratory conditions, (2) grants access to unlimited numbers of (clonal) individuals, (3) is microscopically, molecularly, and genetically tractable, and (4) allows for standardization of protocols, tools, and techniques. Here, we focus on Aiptasia as a coral model organism to elucidate host-microbial interactions and advance microbiome-based interventions (while acknowledging that a number of coral models are being actively developed).

The focus on Aiptasia as a model for coral biology was initially driven by the need for a model organism to unravel cell biological mechanisms related to coral-algal symbiosis (Weis et al. 2008). However, even then, Aiptasia was not new to the field as it had been employed to study cnidarian-dinoflagellate symbioses since the early 1980s (Glider et al. 1980; Gates et al. 1992; Muller-Parker and Davy 2001; Belda-Baillie et al. 2002). Researchers appreciate Aiptasia as it propagates quickly, so much so that it is considered a pest among hobby aquarists (Hunter 1984). Moreover, Aiptasia is easy to maintain in clonal lines, unlocking high-throughput experimental capacities avoiding the cost and logistics of fieldwork as well as the need to sacrifice precious coral biomass (Weis et al. 2008; Voolstra 2013). The molecular resources available for Aiptasia have steadily increased over recent years, now encompassing most methods and techniques readily available in other cell biological model systems. These include a sequenced genome (Baumgarten et al. 2015), published transcriptomes (Lehnert et al. 2012, 2014; Baumgarten et al. 2018), and a recently closed sexual reproductive life cycle under laboratory conditions (Maegele et al. 2023), paving the way for targeted forward and reverse genetic approaches (Jones et al. 2018; Cleves et al. 2020; Roberty et al. 2024). Importantly, and counter to their coral counterparts, Aiptasia engage in facultative symbioses with their algal symbionts. Thus, they can be kept symbiont-free (aposymbiotic), which allows for important control studies to disentangle host from algal symbiont effects (Lehnert et al. 2012; Voolstra 2013; Presnell et al. 2022; Xiang et al. 2022). In addition, protocols to render Aiptasia axenic (i.e., devoid of microbes) or at least gnotobiotic (i.e., the few associated microbes are known and accounted for) are available (Costa et al. 2019, 2021; MacVittie et al. 2023). Importantly, Aiptasia can be maintained in these microbe-free states for months or years and can be re-infected with a variety of Symbiodiniaceae and/or bacteria strains. To provide the foundation for microbiome-based research, the bacteria-carrying capacity and microbiomes of Aiptasia have been described, and bacterial inoculations as well as microbiome transplantations have been attempted (Röthig et al. 2016; Herrera et al. 2017; Costa et al. 2021). Thus, what started as a model organism for coral-algal symbiont cell biology has since grown into a powerful model system poised to advance our understanding of the functional and mechanistic aspects underlying host-microbiome interactions (Dörr et al. 2024) Fig. 13.3).

Characterizing the composition and function of the Aiptasia microbiome is crucial for its utility as a model to develop effective microbial-based therapies. So far, the phylogeny and taxonomy of the microbiomes of Aiptasia strains H2 (Herrera et al. 2017), CC7 (Röthig et al. 2016; Herrera et al. 2017), four GBR strains (Hartman et al. 2020), and a global collection of strains (Brown et al. 2017) have been characterized under culturing conditions using 16S rRNA gene amplicon sequencing. For some strains, the bacterial community has also been described (1) under symbiotic and aposymbiotic states (Röthig et al. 2016; Xiang et al. 2022), (2) under varying environmental conditions following long-term temperature stress (Ahmed et al. 2019; Hartman et al. 2019), (3) after short-term heat stress with and without changing salinities (Randle et al. 2020; Dungan et al. 2021; Sydnor et al. 2023), or (4) from different tissue sections (Maire et al. 2021). In general, Aiptasia strains are associated with 100s of different bacteria, including Alphaproteobacteria (e.g., Rhodobacteraceae, Marinobacter or Sphingomonadaceae), Gammaproteobacteria (e.g., Alteromonadaceae, Pseudoalteromonadaceae, Vibrionaceae), and Flavobacteriaceae, among other families (Röthig et al. 2016; Brown et al. 2017; Herrera et al. 2017; Dungan et al. 2020; Costa et al. 2021). Many of these bacterial families and species were previously found to be associated with corals, sponges, algae, and marine sediments (Röthig et al. 2016).

Due to the complexity of the coral holobiont, understanding the contributions of individual organisms is a major challenge (Jaspers et al. 2019). Consequently, we still lack knowledge of the traits underlying microbes that are suitable probiotics (Schultz et al. 2022). To approach the complexity of coral holobionts, one may follow a reductionist approach employing the Aiptasia metaorganism, by which the individual parts of the disassembled metaorganism (host, algal symbionts, and bacteria) can be used as building blocks to (re-)assemble an experimentally configured holobiont in a controlled mix-and-match manner, much like a construction kit (“Baukasten”). Such an approach requires to culture bacterial isolates from Aiptasia (Röthig et al. 2016; Dungan



Strengths & weaknesses

- Slow growth
- Animals are genetically variable
- Fragments (multiple polyps) assumed to represent colony biology
- Species diversity
- Diversity of growth forms
- Diversity of stress resilience
- Diversity of algal & bacterial symbioses

Investigated microbial interactions

- Prebiotics, postbiotics, probiotics
- Microbiome transplants
- Phage therapy

Investigated microbial interactions

- Independent study of bacteria, algae, and hosts (disassembled metaorganism)
- Targeted study of defined bacterial-algal-host combinations (reassembled metaorganism)
- Manipulative microbiome experiments under different host backgrounds (symbiotic, aposymbiotic, native, axenic)

Strengths & weaknesses

- Available in large numbers
- Clonal animals
- Fast growth
- Simple maintenance
- Closed life cycle (sexual/asexual)
- Single polyp represents whole animal
- Facultative symbiotic with Symbiodiniaceae
- Limited representation of in situ reef environments
- No calcification

Fig. 13.3 The Aiptasia model organism offers a powerful toolbox to untangle microbial interactions. Although a broad suite of approaches is available to study microbial interactions in the coral holobiont, experiments in laboratory settings are challenging. Aiptasia has the advantage of simple maintenance and high-throughput scaling under culture conditions, while its facultative symbiosis with algae allows for targeted interrogations of bacterial and host effects in aposymbiotic or axenic host backgrounds [Photo credits: Christian R Voolstra (Aiptasia) and Anna Roik (*Acropora* coral)] et al. 2021) or coral (Sweet et al. 2021), which can then be tested for function on axenic (gnotobiotic) Aiptasia hosts or against Aiptasia hosts with their resident microbiome intact (i.e., native holobiont). Even though the collection of bacterial isolates from coral and Aiptasia hosts is growing, three problems need to be addressed for the purpose of enhancing the selection of probiotic candidates: (1) increasing the diversity of cultured bacteria, (2) standardizing their screening for a beneficial effect on the host, and (3) identifying beneficial bacterial traits which confidently predict their probiotic potential. Increasing the range of culturable bacteria remains challenging, as many bacteria grow slowly, have unknown nutrient requirements, or need additional cues for successful growth. Thus, recent studies put emphasis on novel isolation and culturing methods (Raina et al. 2009; Keller-Costa et al. 2017; Pogoreutz and Voolstra 2018; Sweet et al. 2021; Schultz et al. 2022; Modolon et al. 2023). Once bacteria are isolated, screening for their beneficial potential is crucial to ensure that non-harmful bacteria with beneficial traits are chosen as probiotic candidates (Box 13.1) (Peixoto et al. 2017). However, most beneficial traits are currently only hypothesized and inferred by means of molecular or physiological assays that reflect trait expression in culture, but rarely in a holobiont background (Santoro et al. 2021). Thus, a definitive list of traits or genes that accurately predict the probiotic potential of a given isolate are currently unavailable. The proof of principle relies on testing probiotic candidates for their beneficial effects on the host under those stress conditions that are sought to be ameliorated, ideally in a standardized and scalable manner. Short-term acute thermal assays using the Coral Bleaching Automated Stress System (CBASS) (Voolstra et al. 2020; Evensen et al. 2023) were recently shown to resolve differences in stress tolerance phenotypes of Aiptasia following bacterial inoculation, and thus, provide such a standardized and reproducible experimental platform to screen bacterial candidates that increase holobiont thermal tolerance and resilience (Dörr et al. 2023). Besides the confirmation of pre-screened bacterial isolates for their probiotic potential, CBASS assays can also be used to identify bacterial isolates with a beneficial effect that can then be interrogated for their underlying traits or functional contribution through, e.g., metagenomic or metatranscriptomic analysis (Fig. 13.3). Thus, new candidate traits or marker genes may be identified. Coral microbiome studies have successfully applied metagenomics (Robbins et al. 2019; Cárdenas et al. 2022; Hochart et al. 2023), with protocols recently being adapted for Aiptasia (Voolstra et al. 2022).

Taken together, Aiptasia is a powerful model organism that allows the targeted (re-)assembly and testing of different metaorganism configurations with subsequent assessment of holobiont phenotypes and the interrogation of mechanistic aspects using metagenomic/metatranscriptomic analysis. In conjunction with standardized screening approaches (e.g., CBASS), the Aiptasia coral model is poised to develop into a scalable high throughput framework to assess and test host microbiome interactions and development of probiotic protocols. Through studying Aiptasia, microbial-based therapies can be refined and contribute to the conservation and preservation of coral reefs globally.

13.7 Conclusion

Here we explored the potential of microbial-based therapies for restoring or rehabilitating compromised coral holobionts, highlighting the intricate relationship between corals and their microbiomes. The use of probiotics, prebiotics, postbiotics, microbiome transplantation, and phage therapy represents a frontier in coral active intervention efforts addressing the urgent need to counteract the detrimental effects of environmental stressors on coral health. Key to the advancement of microbial therapies for corals is understanding under what conditions and at what life stage probiotic therapy will provide optimal results, in addition to the development of efficient delivery methods tailored for reef environments. It is worthwhile to keep in mind that healthy microbiomes may exhibit resistance to alteration and actively managed microbiomes often revert to their original state. Thus, microbial therapies should be used in conjunction with other methods, tailored to the specific need, and generally understood as medicine to support resilience until more permanent solutions are achieved. It is also worthwhile to further study host epigenome changes following microbial-based therapies, which may provide data to support a more stable effect. Although microbial therapy is demonstrated to work in principle, the complexity of microbial interactions and the dynamic nature of the coral microbiome necessitate further research to fully understand the mechanisms underlying its beneficial effects. The Aiptasia model system has proven invaluable for advancing our understanding of coral-microbe interactions, offering a robust clonal coral model organism with which to perform highly controlled, standardized experiments. As a scalable and tractable model, Aiptasia paves the way for high-throughput screening of microbial therapies and provides insights into the broader applicability of these treatments across different coral species and environmental contexts. Continued research and development are crucial for refining microbial-based therapies, ensuring their efficacy and safety, and ultimately deploying them as part of a comprehensive strategy to preserve coral reefs in the face of escalating global environmental challenges.

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14

Considerations, Ethics, and Risk Assessment for the Development and Application of Microbial-Based Therapies; Introducing a Rights-Based Framework for Reef Communities

Emma F. Camp, Genevieve Wilkinson, Paige Strudwick, and David J. Suggett

Abstract

Active intervention is now considered fundamental to support coral reefs as they continue to experience rapid environmental change. A range of interventions are being trialled and implemented that typically have a common goal of increasing coral resilience. Microbial-based therapies have been proposed as an innovative way to support coral fitness and mitigate anthropogenic impacts. In this chapter we explore the risks, caveats, and ethical considerations surrounding the deployment of microbial-based therapies. As coral reefs are socio-ecological systems, we consider these points from both an ecological and societal perspective. We propose using a rights-based approach (RBA) to aid decision-making on the suitability of restoration practices and their associated risks and benefits. Specifically, we introduce the LAPNE framework which considers Legality, Accountability, Participation, Non-discrimination and equality, and Empowerment, and we demonstrate its application to microbial-based therapies. We conclude by summarising how an RBA could broadly benefit all restoration practices by providing a framework to ground human-rights and international obligations as well as providing a legal framework for unified decision making.

Keywords

Active intervention · Coral reef · Microbiome · Restoration rights-based approach · Risk

14.1 Introduction

The unprecedented rate of environmental change and habitat loss has intensified global efforts to restore coral reefs. The 2022 Intergovernmental Panel on Climate Change reported with high confidence that at 1.5 °C warming warm-water coral-dominated systems will largely be non-existent (Hoegh-Guldberg et al. 2022). Given such dire predictions, there is increasing sentiment that immediate interventions are fundamentally required alongside reduced greenhouse gas emissions to ensure a future for coral reefs (Van Oppen and Oliver 2015; Anthony et al. 2017; Anthony et al. 2020; Duarte et al. 2020). Over 200 countries signed the Kunming-Montral agreement in December 2022 that aims to protect or restore at least 30% of all habitats and aligns to the United Nations Decade on Restoration to fast-track recovery of degraded ecosystem health and associated livelihoods. While reef restoration has been occurring in some reef locations for decades (e.g., in the Caribbean; Young et al. 2012), other countries (e.g., Australia; Howlett et al. 2022; McLeod et al. 2022) have only recently adopted restoration as a management tool in response to sudden catastrophic declines in coral cover. This fast-evolving discipline of ecosystem management creates challenges as the science, policy, and ethics must be established to support the intensifying need for restoration (Peixoto et al. 2024b; Anthony et al. 2020; Morrison et al. 2020). Furthermore, the development of coral restoration at scales required to sustain reef functioning presents diverse challenges from approaches used (Suggett and van Oppen 2022) to sustainable financing mechanisms (Suggett et al. 2023). Such challenges stall implementation whilst

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coral reef health continues to decline (GCRMN 2020), meaning that time-critical, novel, and broadly usable solutions are needed (Voolstra et al. 2021).

No single restoration practice can "solve" the coral crisis (see Suggett et al. 2024), but collectively, a toolbox of possible solutions can help buy time for reefs by enhancing their resilience to ongoing environmental challenges (Peixoto et al. 2024a). Coral microbial therapies are one form of active intervention from the restoration toolbox that are being trialled and implemented globally. The goal of microbial therapies is to remedy the shifting baselines (Peixoto and Voolstra 2023) of coral reefs occurring on the microbial scale that have resulted from compounding global pressures (e.g., Santoro et al. 2021; Morgans et al. 2020). Microbial interventions are diverse (e.g., using viruses, bacteria, Symbiodiniaceae) but typically aim to aid coral/ecosystem resilience and immunity by restoring or reinstating 'healthy' coral microbiomes (Peixoto et al. 2017; Doering et al. 2021; van Oppen and Nitschke 2022; Garcias-Bonet et al. 2023). Here we discuss the risks, caveats, and ethical considerations for the implementation of microbial therapies in reef restoration. We next propose that a rights-based approach (RBA) can provide a roadmap for scientists and stakeholders to use when considering the suitability of restoration practices and their associated risks and benefits (Camp et al. 2024). Human rights principles are increasingly being considered in environmental policies and treaties, for example in the Strategic Plan on Biodiversity (2011–2020), Agenda 2030, and the right to a healthy environment (Ituarte-Lima et al. 2019). Human rights are independent but indivisible from environmental protection and have already been agreed upon by most countries, thus providing a normative framework for decision making (Ituarte-Lima et al. 2019). We demonstrate the application of the RBA to microbial-based therapies and outline areas for future consideration.

14.2 Microbiome in Reef Restoration

The microbiome of corals—as with all organisms—is fundamental to healthy functioning and persistence (Bourne et al. 2016). It is an aspect of coral biology that can be directly or indirectly impacted by reef restoration processes (Gantt et al. 2023; Strudwick et al. 2022, 2023, 2024), but can also be manipulated in support of reef restoration goals (e.g., potential enhanced coral fitness; Peixoto et al. 2021). Indirect impacts may carry the greatest risk as they are often unaccounted for until they present, where variability in the coral microbiome often appears unpredictable in nature and differs across species (Voolstra and Ziegler 2020). Changes in coral-associated bacterial communities have been documented during the propagation and/or outplanting stages of reef restoration *in situ* (Great Barrier Reef; Strudwick et al. 2022, 2023, 2024) and changes in Symbiodiniaceae microalgae communities have occurred within Lendo et al., in revision) *ex situ* land-based nurseries (Florida Keys; Gantt et al. 2023). In a restoration context, documented microbiome changes have not (yet) been associated with detrimental impacts to the coral holobiont; however, coral studies transplanting corals between environments have documented increases in pathogenic bacteria (Casey et al. 2015). Our understanding of the impact of restoration practices on other microbial members of the holobiont (e.g., viruses and archaea) is in its infancy and is an important area for future research. Closing such fundamental knowledge gaps is time critical as global pressures threaten the health of coral and coral reef ecosystems on a microbial scale (Peixoto and Voolstra 2023) and we must ensure a holistic approach is applied to safe-guarding the resilience of such ecosystems. Host-associated microbial symbionts are of particular concern due to their vulnerability and sensitivity to environmental change (Gardner et al. 2019; Boilard et al. 2020) and known variability during some restoration practices (Strudwick et al. 2022). There are risks that changing microbiomes during restoration efforts could compound emerging deterioration of microbial health resulting from changing global conditions (Peixoto and Voolstra 2023). Locations targeted for restoration are also typically degraded reef-scapes that can have significantly degraded (dysbiotic) microbial communities and environmental conditions favouring disease-causing organisms (Moriarty et al. 2020). Given the essential role of the coral microbiome in tolerance to environmental change (van Oppen and Blackall 2019), and the further forecasts in deteriorating reef health condition (Sully et al. 2022; Khalil et al. 2023), preservation—or indeed active enhancement—of healthy coral-microorganism associations is required for the persistence of coral reefs into the future. Consequently, to stem fundamental biodiversity losses, considerations of the coral microbiome should be anchored in all future restoration decisions from planning through to implementation, monitoring, and evaluation (Peixoto et al. 2022).

Natural variability of coral microbiomes for some coral species (Ziegler et al. 2017; Haydon et al. 2021; Strudwick et al. 2022, 2023) presents an opportunity for reef restoration practitioners to 'harness the microbiome' to their advantage (Voolstra et al. 2021; Peixoto et al. 2022). The enrichment of putatively Beneficial Microorganisms for Corals (pBMC) (Peixoto et al. 2017) via the application of 'probiotics' (Rosado et al. 2019) or the introduction of heat tolerant symbiotic microalgae (Buerger et al. 2020) upon propagated and/or outplanted corals could enhance coral survival and boost restoration success (Peixoto et al. 2021). Alternate treatments may not involve the introduction of beneficial microorganisms but rather mitigate putatively harmful microorganisms that may enhance coral health and consequently restoration success. For example, the modulation of pathogenic bacteria with viruses via 'phage therapy' (van Oppen and Nitschke 2022) and topical antibiotic treatments to reduce disease occurrence which have been successfully applied *in situ* (Forrester et al. 2022; Neely et al. 2020). Similar microbiome manipulations have improved outcomes in terrestrial agriculture (Foo et al. 2017) and aquaculture (Khati et al. 2018), hence they may provide an opportunity to reduce disease, enhance stress tolerance and provide nutritional advantages (Thatcher et al. 2022) for corals during reef restoration. Delivery strategies for probiotics in marine environments include direct inoculation with cultures of free-living cells (Rosado et al. 2019), inoculation of carrier materials (Gao et al. 2020; Qiao et al. 2020), suspension in saline solutions (Delgadillo-Ordoñez et al. 2024), bioencapsulation within live food (Van Hai et al. 2010; Assis et al. 2020), and inoculation of biopolymers for delivery to specific animal compartments (Rosas-Ledesma et al. 2012). Successful applications of microbiome restoration in corals *ex situ* are more comprehensively discussed at Chap. 13. Overall, these efforts include direct inoculation of pBMC bacterial isolates via enriched sea water to provide improved energy reserves and rates of calcification (Zhang et al. 2021), thermal tolerance (Santoro et al. 2021) or mitigate the impacts of hydrocarbon contamination (Villela et al. 2023) and reintroduction of selectively bred 'heat-evolved' Symbiodiniaceae strains into coral larvae to provide enhanced thermal resilience (Buerger et al. 2020). Phage therapy by delivery of 'phage cocktails' has also been successfully applied to inhibit the activity of *Vibrio coralliilyticus*—the etiological agent of bacterial coral bleaching (Ben-Haim et al. 2003)—proving the suitability of phage therapy for treatment of coral disease (Cohen et al. 2013). Applications of microbiome restoration *in situ* are increasing, with examples including the grafting of phenotypically distinct coral fragments to successfully provide altered disease resistance and bleaching susceptibility (Rosales et al. 2019), inoculation of corals with pBMC's (Delgadillo-Ordoñez et al. 2024), microbiome transplantations (Doering et al. 2021) and delivery of antibiotics via topical pastes (Forrester et al. 2022; Neely et al. 2020). While application of microbiome manipulation *ex situ* (in aquaculture facilities) to produce resilient individuals for reef-restoration hold promise, urgent research and development, and a science-based framework for the scaled *in situ* application of probiotics should be used for real world reef restoration (Peixoto et al. 2022).

14.3 Considerations, Caveats, and Risk for Microbial-Based Therapies

Actioning interventions, such as reef restoration, to protect or rehabilitate vulnerable ecosystems inherently comes with a wealth of considerations and risks (Mcleod et al. 2019; Anthony et al. 2020). However, the alternative risks of inaction condemn these ecosystems and society to irreversible losses in resilience and biodiversity, ultimately diminishing any chance of a future for coral reefs (Peixoto and Voolstra 2023). Hence, society is no longer faced with the question of *whether* reef restoration should be applied but only *when, where,* and *how* it should be implemented (Suggett et al. 2024). A key step in the selection and application of microbial-therapies links to the risks associated with them and how they can be mitigated or objectively rationalised. Risks associated with microbiome shifts relate to both society and the environment and from an individual to global scale (Fig. 14.1a). For example, risks to the individual target coral include incompatibility between novel and native consortia resulting in dysbiosis (Peixoto et al. 2022) and eventually causing more harm than good to holobiont fitness. Additionally, functions for many coral-associated microorganisms remain unresolved (Peixoto et al. 2017) and while certain taxa may impart beneficial functions during *ex situ* testing (or in culture) their functioning may vary *in situ* and/or *in hospite* (in response to different prevailing environmental conditions) leading to unforeseen impacts to the coral holobiont or broader ecosystem (Voolstra et al. 2021); this is particularly the case under ocean warming as this is known to trigger pathogenic microbial proliferation (Moriarty et al. 2020). Further, specific mechanisms underpinning interactions between microorganisms within the coral holobiont are also unresolved (Peixoto et al. 2017; Sweet and Bulling 2017); therefore, we cannot accurately predict the outcomes of microbiome manipulation on the remainder of the microbiome.

Importantly, the microbial baseline for reefs are already shifted from historic 'pristine reef' that may influence reef resilience (Peixoto and Voolstra 2023). Within this context, the risk of no intervention must be considered. Furthermore, propagation and out-planting restoration interventions also have the potential to impact coral microbiomes (Casey et al. 2015; Moriarty et al. 2020; Strudwick et al. 2022, 2023) therefore risks are not limited to microbiome restoration but rather interventions as a whole. The risks of microbiome interventions can be minimised if an ecological restoration or rehabilitation approach is adopted where, for example, only commonly found native consortia are applied or restored, rather than more exploratory innoculations on healthy individuals with non-native microorganisms (Contos et al. 2021). In plant biology, transplantation of entire soil microbes have been undertaken to support ecosystem "rewilding" (Lance et al. 2019), and coral microbiome transplantation has also been successfully undertaken (Doering et al. 2021). To truly understand the risks associated with *in situ* application of microbial restoration it is essential for pilot studies to be conducted *in situ*. Stringent permitting risk assessment processes restrict the advancement of knowledge required for pragmatic application of microbiome restoration. Delgadillo-Ordoñez et al. (2024) recently conducted a detailed risk assessment of *in situ* probiotic application highlighting minimal impacts to bacterial communities of surrounding seawater and sediments post-inoculation which suggests the promising targeted effect of their approach and provides a template for future *in situ* risk assessments or pilot studies.

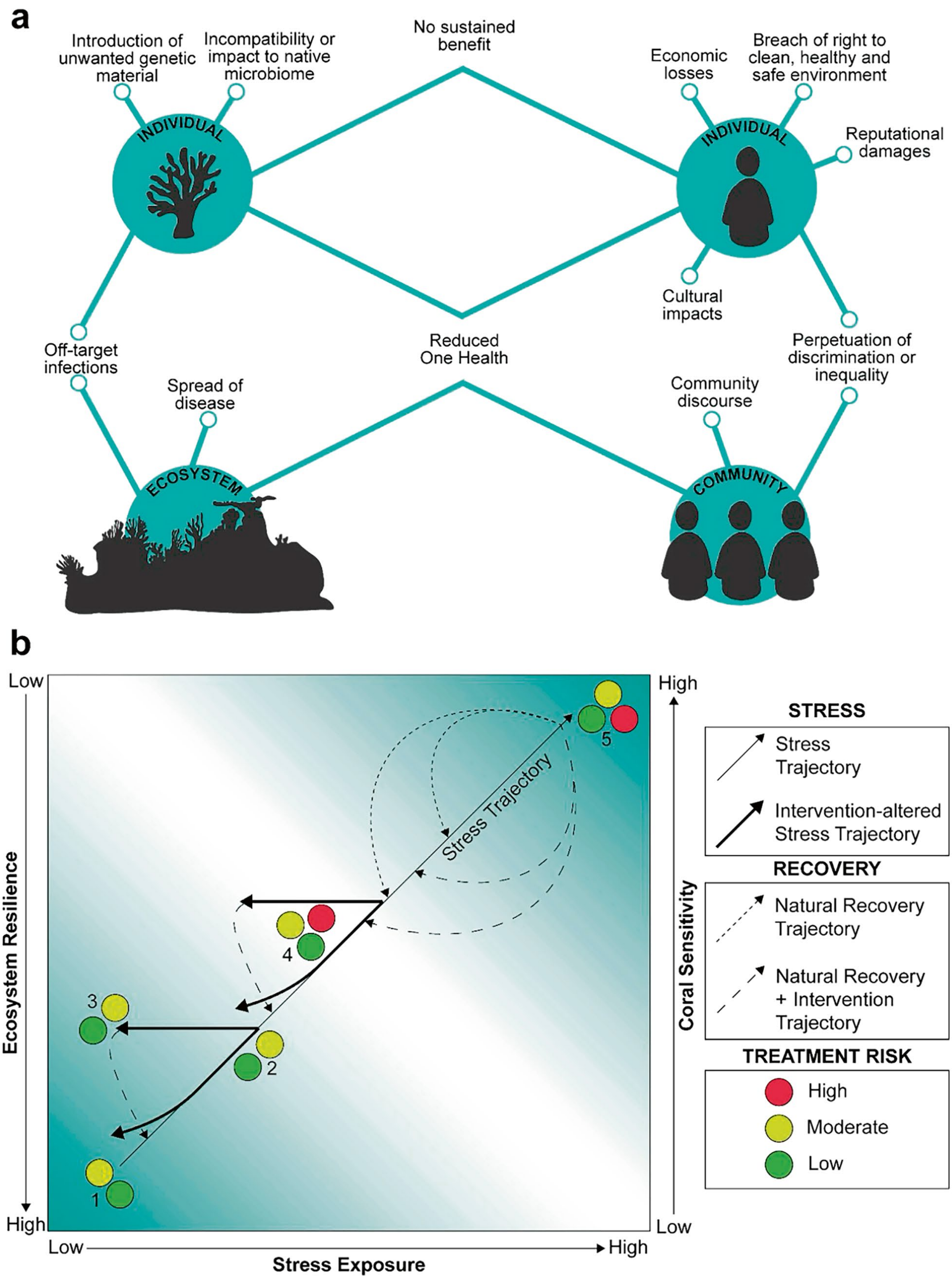
a
Introduction of unwanted genetic material
Incompatibility or impact to native microbiome
No sustained benefit
Economic losses
Breach of right to clean, healthy and safe environment
INDIVIDUAL
INDIVIDUAL
Reputational damages
Cultural impacts
Off-target infections
Reduced One Health
Perpetuation of discrimination or inequality
Spread of disease
Community discourse
ECOSYSTEM
COMMUNITY
b
Low
High
Ecosystem Resilience
Stress Trajectory
1
2
3
4
5
Low
High
Stress Exposure
High
Low
Coral Sensitivity
STRESS
Stress Trajectory
Intervention-altered Stress Trajectory
RECOVERY
Natural Recovery Trajectory
Natural Recovery + Intervention Trajectory
TREATMENT RISK
High
Moderate
Low

Fig. 14.1 Risk considerations for microbial-based therapies. (**a**) A schematic highlighting that risks associated with microbiome manipulation relate to both society and the environment, and from an individual to global scale. Environmental and societal risks are often interconnected, with both types of risk important to consider when evaluating the suitability of an intervention. (**b**) Adapted from Camp (2022) and Dawson et al. (2011) the graph illustrates a consideration between ecosystem resilience, stress exposure and coral sensitivity over when and what microbial-based therapies could be implemented to minimise risk. The left Y-axis considers the ecosystems resilience to environmental change, while the right Y-axis accounts for the coral's sensitivity. Sensitivity is shaped by endogenous factors and includes evolutionary potential, phenotypic plasticity, growth capacity, biological interactions that impact fitness and external drivers. Under stress (X-axis) the reef trajectory declines (represented by the thin solid diagonal arrow), but with intervention the stress trajectory could be altered; theoretical changes in trajectory are shown in bold arrows. Interventions could also augment recovery as illustrated by the dashed lines. The timing of when a given intervention is deployed should account for the type of intervention, the reef state and the risk associated with the intervention. Possible scenarios: (*1*) a low or moderate risk microbial-based therapy could be deployed prior to any stress to augment resilience. (*2*) Early in a stress event, a low or moderate risk microbial-based therapy could be deployed with the goal to halt the increasing stress trajectory, and either sustain or enhance the system's resilience. (*3*) Complimentary to scenario 2, low or moderate risk treatments could be further employed to augment natural recovery. (*4*) Under severe stress, with lowering ecosystem resilience and increasing coral sensitivity, higher risk microbial therapies could be deemed appropriate. As with scenario 2, the goal would be to alter the stress trajectory and enhance natural recovery. (*5*) Some microbial-based therapies may only be suitable after a stress event, or the stress event could occur before they can be deployed. In these scenarios the hope would be to enhance natural rates of system recovery. Notably, moderate to high (or even low) risk interventions may be suitable if actions are taken to mitigate or minimise risks

Accompanying all risks to the ecosystem are associated risks to society. Economic risk is a major factor, where investments return no benefit when application of microbiome manipulation is unsuccessful or produces unsought outcomes, e.g., if the location of the beneficial bacterial does not align with the location their function is required (van Oppen and Nitschke 2022) or there is limited temporal stability. Further, in the worst-case scenario microbiome manipulation leads to detrimental outcomes that require 'clean-up' efforts and associated costs to rectify damages, if even possible. Risks to the ecosystem range from high to low depending on the initial state of the reef (health and resilience) and the mode of microbiome manipulation employed (Fig. 14.1b). Societal risks also range from high to low and from the individual applying the innovative approach to the wider community. Beyond the economic context, societal risks include limited accessibility; for example, community-led reef restoration efforts in low economy nations where required infrastructure (e.g., laboratories or microbiological equipment) may not be available (Weeks and Adams 2018) and hence would be disadvantaged. There are also risks that restoration efforts will disadvantage minority groups and contribute to inequality (Cruz-Alonso et al. 2023; Toone et al. 2022). In freshwater ecosystem restoration, a lack of consultation, disproportionate share of environmental hazards, and practitioners celebrating macro-economic success rather than direct benefits to society all contributed to inequity of minority groups (Díaz-Pascacio et al. 2022). There are also risks to culture and to First Nations peoples when Western scientific practices are used that may be in conflict with local traditional practices and thus ensuring co-design with traditional reef owners and stakeholders is critical to reduce societal risks (Gibbs et al. 2021). These risks engage states' obligations to respect, protect and fulfil human rights, particularly economic, social and cultural rights protected by the International Covenant on Economic, Social and Cultural Rights such as rights to an adequate standard of living and to take part in cultural life and the emerging human right to a clean, healthy and sustainable environment.

The risks highlighted here are not exhaustive, and some risks will not be predictable. The risk landscape is dynamic and dependent on the environmental state, risk to society, the invention type and severity of stress (Fig. 14.1). A challenge for restoration practitioners is thus deciding when a given intervention is appropriate while also accounting for uncertainty. Current efforts to guide these decision-making processes include the use of modelling and digital-twins, diverse consultation groups, research data, and decision-making tools e.g., Comprehensive Assessment of Risk to Ecosystems (CARE) (Battista et al. 2017; Vougler 2022; Sleeter et al. 1983; Yuen et al. 2023; Anthony et al. 2020) and the use of nature-based microbial stewardship. In this case, a possible solution to minimise such risks is to use microbiome restoration and rehabilitation, rather than random manipulation, aiming at restoring native, common (but also sensitive to stress) marine mutualistic members of the microbiome (Peixoto et al. 2022; Peixoto and Voolstra 2023). In the next section, we introduce a rights-based approach (RBA) to reef restoration and discuss how it can aid decision making and risk management.

14.4 Introducing a Rights-Based Approach (RBA) to Coral Restoration

A human rights-based approach to environmental protection considers the protective actions against key human rights and environmental legislation and has been proposed as an effective way to build climate resilience, utilise nature-based solutions and to meet the needs of societies while addressing fundamental inequalities that prohibit progress (United Nations Environment Programme 2022). The 2015 adoption of the United Nations Sustainable Development Goals by over 193

Member States reinforced the interlink between human and environmental health, stating that “(t)hey are integrated and indivisible and balance the three dimensions of sustainable development: the economic, social and environmental” (United Nations 2015). In 2022 the United Nations General Assembly declared everyone has a right to a clean, healthy and sustainable environment (United Nations 2022), which further supports an approach that considers both human rights and broader environmental rights. Coral reefs are considered socio-ecological systems (SES), with SES frameworks suggested for restoration that help account for both the environmental and social elements within restoration activities (e.g., Uribe-Castañeda et al. 2018. Suggett et al. 2023). Here, we propose a rights-based approach (RBA) framework for assessments of reef restoration activities (Camp et al. 2024) that can be complementary to an SES framework. The RBA is grounded in human rights and international obligations that provide a benchmark for unified decision making. Where there is fragmentation between human rights and environmental obligations developed outside of a human rights framework, the key objective is systemic integration so that principles are interpreted harmoniously (International Law Commission 2006).

An RBA to coral reef restoration provides a means to consider legality, accountability, non-discrimination and equality, participation and engagement. It recognises that to be achievable, coral reef restoration relies on both international and national laws, as well as stakeholder buy-in. Using an RBA could help frame multiple socio-ecological aims to define restoration objectives ultimately needed to evaluate success (Anthony et al. 2020), while also managing risk by considering both societal and environmental needs. In proposing the use of an RBA, we do not suggest that protection of the environment for its own sake is not important and agree ‘that the objective must be to improve the quality of the environment and not just to exploit it for human rights’ (Odote 2020). However, we argue that this protection should be consistent with international human rights obligations as ‘the interests and duties of humanity are inseparable from environmental protection’ (Borràs 2016). Principles developed by the Special Rapporteur on Human Rights and the Environment shape our proposed RBA (Knox and Boyd 2018; Table 14.1) and are referred to at relevant sections below. These principles establish substantive and procedural elements of the right to a healthy environment; for example,

Table 14.1 The 16 principles developed by the Special Rapporteur on the human right to a clean, healthy and sustainable environment. Next to the framework principles we map the relevant LAPNE principles that need to be considered within reef restoration

Framework principle	Consideration within a reef restoration perspective; Relevant LAPNE principles
Framework principle 1—states should ensure a safe, clean, healthy and sustainable environment in order to respect, protect and fulfil human rights.	LEGALITY ACCOUNTABILITY
Framework principle 2—states should respect, protect and fulfil human rights in order to ensure a safe, clean, healthy and sustainable environment.	LEGALITY ACCOUNTABILITY
Framework principle 3—states should prohibit discrimination and ensure equal and effective protection against discrimination in relation to the enjoyment of a safe, clean, healthy and sustainable environment.	NON-DISCRIMINATION AND EQUALITY ACCOUNTABILITY
Framework principle 4—states should provide a safe and enabling environment in which individuals, groups and organs of society that work on human rights or environmental issues can operate free from threats, harassment, intimidation and violence.	EMPOWERMENT PARTICIPATION
Framework principle 5—states should respect and protect the rights to freedom of expression, association and peaceful assembly in relation to environmental matters.	EMPOWERMENT PARTICIPATION

(continued)

Table 14.1 (continued)

Framework principle	Consideration within a reef restoration perspective; Relevant LAPNE principles
Framework principle 6—states should provide for education and public awareness on environmental matters.	ACCOUNTABILITY EMPOWERMENT PARTICIPATION
Framework principle 7—states should provide public access to environmental information by collecting and disseminating information and by providing affordable, effective and timely access to information to any person upon request.	ACCOUNTABILITY EMPOWERMENT PARTICIPATION
Framework principle 8—to avoid undertaking or authorizing actions with environmental impacts that interfere with the full enjoyment of human rights, states should require the prior assessment of the possible environmental impacts of proposed projects and policies, including their potential effects on the enjoyment of human rights.	ACCOUNTABILITY PARTICIPATION
Framework principle 9—states should provide for and facilitate public participation in decision-making related to the environment, and take the views of the public into account in the decision-making process.	ACCOUNTABILITY EMPOWERMENT PARTICIPATION
Framework principle 10—states should provide access to effective remedies for violations of human rights and domestic laws relating to the environment.	ACCOUNTABILITY PARTICIPATION
Framework principle 11—states should establish and maintain substantive environmental standards that are non-discriminatory, non-retrogressive and otherwise respect, protect and fulfil human rights.	NON-DISCRIMINATION AND EQUALITY ACCOUNTABILITY
Framework principle 12—states should ensure the effective enforcement of their environmental standards against public and private actors.	LEGALITY ACCOUNTABILITY
Framework principle 13—states should cooperate with each other to establish, maintain and enforce effective international legal frameworks in order to prevent, reduce and remedy transboundary and global environmental harm that interferes with the full enjoyment of human rights.	LEGALITY ACCOUNTABILITY
Framework principle 14—states should take additional measures to protect the rights of those who are most vulnerable to, or at particular risk from, environmental harm, taking into account their needs, risks and capacities.	EMPOWERMENT PARTICIPATION

(continued)

Table 14.1 (continued)

Framework principle	Consideration within a reef restoration perspective; Relevant LAPNE principles
Framework principle 15—states should ensure that they comply with their obligations to indigenous peoples and members of traditional communities, including by: Recognizing and protecting their rights to the lands, territories and resources that they have traditionally owned, occupied or used. Consulting with them and obtaining their free, prior and informed consent before relocating them or taking or approving any other measures that may affect their lands, territories or resources. Respecting and protecting their traditional knowledge and practices in relation to the conservation and sustainable use of their lands, territories, and resources. Ensuring that they fairly and equitably share the benefits from activities relating to their lands, territories, or resources.	ACCOUNTABILITY PARTICIPATION NON-DISCRIMINATION AND EQUALITY
Framework principle 16—states should respect, protect and fulfil human rights in the actions they take to address environmental challenges and pursue sustainable development.	ACCOUNTABILITY

Framework Principle 1 that 'States should ensure a safe, clean, healthy, and sustainable environment in order to respect, protect and fulfil human rights' exemplifies the way that these principles safeguard a human rights approach from becoming a vehicle to justify environmental degradation by linking a healthy environment to realisation of human rights (Table 14.1). Principle 13 further requires states to 'cooperate with each other to establish, maintain and enforce effective international legal frameworks to reduce and remedy environmental harm that interferes with the full enjoyment of human rights' (Table 14.1).

An RBA uses key human rights principles and environmental legislation to assess restoration activities and to monitor their effectiveness and compliance, which in turn minimises risk. As human rights have been agreed by most nations, an RBA framework provides a common baseline by which to evaluate coral restoration. We suggest the application of a "PANEL"-based RBA framework to coral restoration as has been previously applied by national human rights institutions (HRTF 2021). The PANEL framework is used to ensure activities are consistent with five key principles: Participation, Accountability, Non-discrimination and equality, Empowerment, and Legality. For the purposes of coral restoration, we propose a reordering of the PANEL principles using a LAPNE approach so that relevant legal principles are understood and identified first, then accountability, participation, non-discrimination, equality, and empowerment considerations can be structured around these obligations. Legality and the regulatory process are considered critical in determining the feasibility of reef restoration (Fidelman et al. 2019; Morrison et al. 2020) supporting this re-ordering. Below we step through the LAPNE framework and provide key questions that would be considered and addressed when applying LAPNE to microbial-based therapies.

14.4.1 Legality

Feasibility for coral reef restoration to be undertaken is (and will likely continue to be) largely governed by regulatory processes (Fidelman et al. 2019). Legality and regulatory processes have the capacity to impact coral restoration positively or negatively. For example, regulations can support efficient, cohesive, and coordinated activities (Fidelman et al. 2019). Conversely, complex legislation and regulatory processes that lack coordination can confuse and challenge restoration efforts (Shumway et al. 2021; McLeod et al. 2018). The evolving nature of reef restoration practices are also going to require regulatory processes to be adaptive (Morrison et al. 2020), which links to considerations of accountability (see section below). Within an RBA framework, a legality assessment focuses on a reef intervention's consistency with relevant environmental and human rights principles, including rights to benefit from applications of scientific progress and protection of the moral and material interests of authors of scientific productions, as well as rights to a healthy environment. Other human rights could be relevant, depending on the nature and impact of the restoration, if, for example, the restoration activity changes the nature of a reef in a way that impacts on the human right to the highest attainable standard of physical and mental health. Rights of cultural minorities may also be engaged if the proposed interventions infringe on their right to enjoy and undertake their cultural practices. Legality requires states to '[recognise] human rights and freedoms as legally enforceable entitlements' (HRBA 2023).

An RBA should establish benchmarks to ensure regulatory processes are consistent with human rights and other international and national obligations relevant to environmental protection and sustainability. Reef restoration activities would be measured against framework principles for a right to a healthy environment, including Framework Principle 2 that 'States should respect, protect and fulfil human rights in order to ensure a safe, clean, healthy and sustainable environment' (Table 14.1). This principle provides focus for the RBA to ensure recognition of specific human rights obligations such as the right to health but is also a vehicle to integrate other legal obligations relevant to reef restoration. Measures of what a clean, healthy and sustainable environment should be can be drawn from standards found in international agreements (e.g., the UN Sustainable Development Goals and High Seas Treaty), international laws (e.g., Convention on International Trade in Endangered Species of Wild Fauna (CITES)) and national or regional legislative processes (e.g., local permitting and workplace health and safety) in a systematic way so that obligations are interpreted consistently with human rights. As Fidelman et al. (2019) highlight for the Great Barrier Reef, the regulatory landscape for restoration is extremely complex, as efforts attempt to coordinate disjointed policies, including managing different values of stakeholders and Traditional Owners (Quigley et al. 2022). Environmental impact assessments consistent with these standards are important components for understanding whether activities facilitate a safe, clean, healthy and sustainable environment and should be central to an RBA.

Using an RBA can also provide assessment benchmarks in the absence of detailed agreement about reef restoration in international law, consistent with the need for dynamic innovation in the field. The pressures of marine ecosystems are catalysing development of innovative science to retain and enhance resilience of these vulnerable ecosystems (not only in the present but to future environmental challenges too), e.g., the development of probiotics and microbiome engineering (Voolstra et al. 2021). Ultimately, this should be addressed through international agreement, consistent with Principle 11 requirements for states to 'establish and maintain substantive environmental standards that are non-discriminatory, non-retrogressive and otherwise respect, protect and fulfil human rights' (Table 14.1).

Example Questions Relevant to a Legality Analysis for Microbial-Based Therapies

What human rights are impacted by the microbial-based restoration activities?

LEGALITY

What human rights are impacted by already degraded microbiomes of coral and reef ecosystems that threaten their existence and the potential inaction allowing their further degradation? Are the microbial-based therapies consistent with these human rights and the framework principles for a right to a healthy environment?

Are microbial-based therapies consistent with international environmental standards that guide our understanding of what a safe, clean, healthy and sustainable government is?

What local, national and international legislation, and traditional owner consent is required for the application of the microbial-based therapies?

14.4.2 Accountability

Once relevant environmental and human rights benchmarks and legislation are identified in the preceding legality analysis, accountability requires effective monitoring of these regulations and remedies for any breaches (HRBA 2023). This aligns with risk management approaches and requires transparency about the expected and actual impact of interventions, as well as the development of human rights indicators to facilitate assessment. Accountability requires that restoration activities have clear objectives and measurements of success for ecosystem function and composition as well as social goals (Hallett et al. 2013; Gann et al. 2019). Embedding human rights benchmarks and indicators into self-assessment procedures for regulatory bodies most closely associated with reef restoration, including the use of environmental impact statements, could strengthen accountability, but also provide a safeguard to ensure broad stakeholder engagement is undertaken. To guarantee stakeholders can participate to ensure accountability, information on the proposed intervention should be translated into meaningful information about possible environmental impacts (and benefits) and potential effects on the enjoyment of human rights, placed into context. Part of this communication may ultimately require evidence from laboratory testing to better inform impact forecasting.

As reefs degrade and as new innovative technologies (such as microbiome manipulation) become readily available, reef managers will have to make decisions on if, when, where, and how they deploy such interventions. This requires consideration of how the decision-making process is undertaken, challenged, implemented, and, ultimately, monitored long-term, reported, and communicated. Effectiveness of the proposed intervention feeds into accountability, and thus rigorous testing, evaluation and improvements that may include 'learn by doing' (Quigley et al. 2022) is important. For reef restoration, to assess effectiveness will typically take years, particularly if there are goals to increase and/or sustain critical reef functions. Consideration of how monitoring would be achieved and ultimately financed for both environmental and rights-based objectives should be undertaken during the project planning phase (Fig. 14.2). Further, how monitoring data is broadly shared to provide collective learning should also be considered. To this point, an outcome of the 15th meeting of the Conference of the Parties for the Conservation of Biodiversity was a proposed voluntary peer review process to accelerate learning through the sharing of knowledge and to support transparent accounting to enhance implementation of the Kunming-Montreal Global Biodiversity Framework (VPR 2023). Ultimately, if an intervention is not achieving human or environmental rights objectives, it needs to be reconceived to ensure that resources are being expended effectively. Standards will need to be enforced (see Principle 12, Table 14.1) and, if breaches occur, a mechanism for redress is necessary (Fig. 14.2), consistent with the obligation to provide remedies for violations of human rights (see Table 14.1; Principle 10). In some states, constitutional rights have helped to provide this redress (e.g., in Kenya and South Africa; Odote 2020), while in countries without constitutional human rights protection this may be achieved through actions available to ensure government accountability in state human rights charters (Clark and Goldblatt 2023).

Example Questions Relevant to an Accountability Analysis for Microbial-Based Therapies

Is the body responsible for RBA of the microbial-based therapies transparently conducting ongoing monitoring (that is not unnecessarily restrictive or prohibitive of progress) to ensure compliance with human rights and environmental benchmarks and review effectiveness of activities?

ACCOUNTABILITY

What accountability mechanisms permit checks and balances on compliance by third parties and are they accessible to stakeholders?

Is microbial-therapy the most suitable option based on the restoration goals? And have these goals been clearly set for both the ecosystem and society?

What are the risks of the microbial-therapy perceived to be? And based on these risks, what is the mode, method, and timing of delivery?

Has the proposed microbial-based therapy had a track-record of success in laboratory testing? If yes, are there any environmental factors (e.g., high water current or type of substrate) that could limit successful application?

How will the therapy be funded, and what is the plan to share results and any intellectual property?

What is the decision-making process going to be like?

How will success be evaluated?

How will complaints be managed?

Have the key stakeholders and Traditional Owners of the site identified for microbial-therapy deployment been consulted to ensure their participation, from design to implementation?

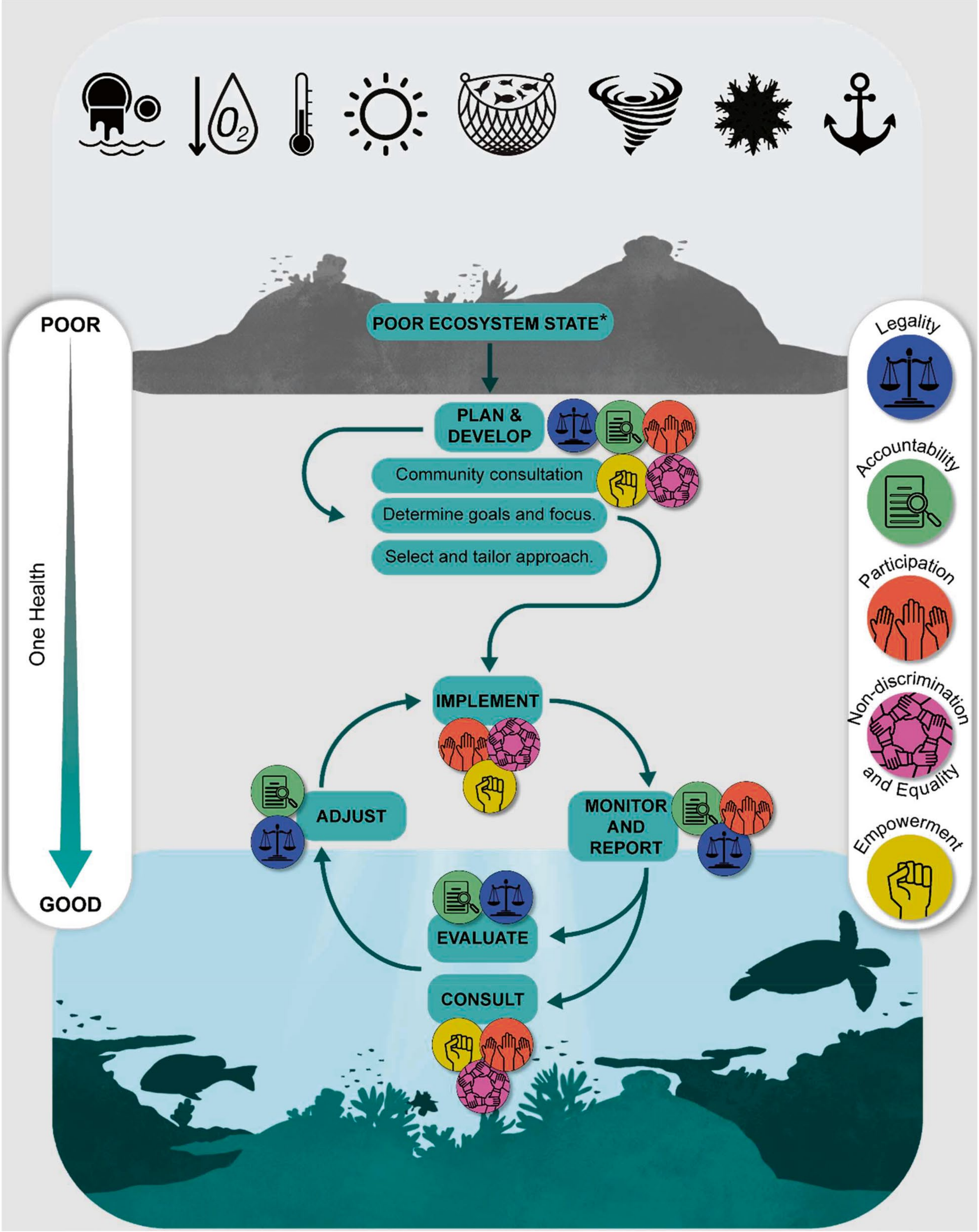

Fig. 14.2 Schematic of the application of the rights-based approach framework, LAPNE to coral reef restoration. Changing environmental conditions indicated by black icons at the top impact the ecosystem to a degrading state, triggering a decision to apply some form of active intervention. From inception, LAPNE principles should be considered and include assessment of legality, accountability, participation, non-discrimination and equality, and empowerment. These principles are applied through the consultation and goal setting phase, implementation, monitoring, reporting, evaluation, and adjustment phases of the project. Icons for each of the LAPNE principles are mapped to stages of the restoration and intervention process. The LAPNE framework embeds human rights and environmental rights and legislation throughout the restoration process and complements a One Health approach to restoration

14.4.3 Participation

Individuals have the right to participate freely, actively, and meaningfully in decisions about their rights (HRBA 2023). Where reef interventions are relevant to rights such as the right to a healthy environment, states should provide mechanisms for participation. Meaningful participation aligns with Principle 9 (see Table 14.1) that states have an obligation to 'provide for and facilitate public participation in decision-making related to the environment and take the views of the public into account in the decision-making process.' General public consultations about changes to policy or law should be augmented with targeted consultation of stakeholder groups and transparent identification of their relevant interests. Priority should be given to groups vulnerable to any negative impacts from the application of the reef restoration approaches, consistent with Principle 14 requirements for states to 'take additional measures to protect the rights of those who are most vulnerable to, or at particular risk from, environmental harm, taking into account their needs, risks and capacities' (see Table 14.1). Priority should also be given to groups with significant cultural connection to the areas in question, such as Traditional Owners. Principle 15 recognises obligations to indigenous peoples and members of traditional communities that can be relevant to reef restoration activities (Table 14.1). Furthermore, as reefs degrade, their ecological, economic and social values reduce, in contrast to restored reefs whose value is likely to increase; this has the potential to create conflict between reef stakeholders, particularly when restoration projects are managed by Western nations based on property-right regimes, in developing nations where traditional management is typically applied (Gibbs and Newlands 2022). While coral restoration can present societal right-based challenges (Gibbs and Newlands 2022) it inherently provides socio-ecological benefits (Suggett et al. 2023), especially where restoration is often initiated through social enterprise (Hein et al. 2019).

Accessibility is a central feature of meaningful participation which requires information to be provided in a comprehensible form and language, consistent with transparency considerations enshrined in Framework Principle 7. This may require the translation of technical environmental assessments into accessible formats. An important tool for understanding the interests of those impacted by interventions is empowerment for individuals and communities so that they can understand their rights and participate in policy development and implementation processes. Resource and intellectual property sharing is also required to ensure non-discrimination and equity of participation (see below). Through participation, broad acquisition of diverse values can be obtained and benchmarked relative to both environmental and rights-based attributes, which can help capture cultural values that are often unaccounted for in monetary valuation-based frameworks (Suggett et al. 2023).

Example Questions Relevant to a Participation Analysis for Microbial-Based Therapies

Who are the key stakeholders and Traditional Owners for the reefs where the microbial-based intervention will be undertaken?

Have they been meaningfully consulted in the design and implementation of the proposed restoration activities?

If the microbial-based agents originated from corals from a different reef site, have the traditional owners there been consulted to understand the transfer of materials between sea counties?

14.4.4 Non-Discrimination and Equality

Discrimination is not permitted in the realisation of human rights and Principle 3 of the human right to a clean, healthy and sustainable environment further provides for states to ensure 'equal and effective protection against discrimination in relation to the enjoyment of a safe, clean, healthy and sustainable environment' (Table 14.1). Safeguarding against discrimination requires identification of vulnerable groups who may be impacted by the relevant intervention. Involving these groups in participation (see above) is one strategy to reduce discrimination. It has been shown that in research, outreach and practice of ecosystem restoration, gender bias and discrimination against marginalised groups persists (Cruz-Alonso et al. 2023; Toone et al. 2022). Within reef restoration, efforts must therefore be made to ensure equality, not only of marginalised groups but with regards to knowledge and resource access. A RBA can provide awareness and therefore consideration on topics such as intellectual property rights, recognising that the net benefits of intellectual property protection can be distributed unevenly and to the disadvantage of low income countries (Yu 2021). Consideration of rights to benefit from the applications of science can help guide knowledge sharing activities (UNHROHC 2020). These approaches also align with obligations protecting traditional knowledge so that the human rights of Traditional Owners are respected, consistent with article 31 of the United Nations Declaration on the Rights of Indigenous Peoples. Environmental treaties also define the necessary for equity, for example, the Nagoya Protocol—Convention on Biological Diversity has an objective for "the fair and equitable sharing of the benefits arising from the use of genetic resources”. Consequently, having an RBA framework for reef restoration (Fig. 14.2) that embeds consideration of non-discrimination and equality can help to achieve both human rights and environmental regulations.

Example Questions Relevant to Non-discrimination and Equality Analysis for Microbial-Based Therapies

Do microbial-based therapies negatively impact vulnerable populations?

NON-DISCRIMINATION
AND EQUALITY

Are measures in place that address inequalities, particularly in relation to access to knowledge?

14.4.5 Empowerment

Empowerment requires that individuals and communities can understand their relevant rights. This can include public education about the nature of a right to a healthy environment, recognising the emerging nature of the right, but also about approaches used in reef restoration. Framework Principle 6 requires states to ‘provide for education and public awareness on environmental matters.’ This should include translating scientific publications into material that the public can understand. Some groups may need special support and recognition. Adolescents have been key activists about preventing environmental harm (Thackeray et al. 2020; Clark and Goldblatt 2023) and should be empowered to participate in consultation processes. The Convention on the Rights of the Child provides children with a right to be heard and requires that the best interests of the child be a primary consideration in all decisions concerning them. Special support could extend to providing resources that empower reef communities by providing access to independent environmental experts who can educate interested parties about the nature of reef restoration activities or legal professionals who can support the use of accountability mechanisms outlined above.

Example Questions Relevant to Empowerment Analysis for Microbial-Based Therapies

Are key stakeholders supported to participate in consultation activities and mechanisms for accountability?

EMPOWERMENT

Is information about the microbial-based therapies, including environmental impact, transparent?

Are educational programs in place to strengthen understanding of these activities and their impact on the environment and any human rights?

14.5 Conclusion

Ongoing environmental change that is driving rapid deterioration of ecosystem health is going to necessitate diverse active interventions for coral reefs that is likely to include microbial-based therapies. Application of these interventions is not without risk, but these risks should be considered relative to the risk of no intervention at all. As coral reefs are socio-ecological systems, the risks and implications of applied interventions need to consider the interconnected ecological and societal impacts arising from their use. In this chapter we have introduced a rights-based approach (RBA) to coral reef restoration that can be broadly applied across interventions. As the right to a safe, clean, healthy, and sustainable environment develops as a human right, using an RBA to consider reef restoration provides a normative framework for decision making across nations. By applying the LAPNE-based RBA framework to microbial-based therapies we demonstrate that consideration of legislative, accountability, participation, non-discrimination and equality, and empowerment at all stages of the restoration process can help to meet all human rights-based obligations while also ensuring nature-centred benefits are achieved. Environmental protection has already been facilitated by a unified health and environment approach; for example, the 'One Health' approach (Dye 2022), where a cross-disciplinary view to improve human health examines the human, animal, and environmental interface. A One Health approach has been proposed for microbial-based therapies (Peixoto et al. 2022) making it well aligned to the LAPNE-based RBA framework. Ultimately there are likely to be ongoing challenges for reef managers to decide when, where, and how to apply reef interventions. However, these challenges carry the risk of leading to inaction, which could be the biggest risk of all. It is therefore time-critical that tools such as RBA frameworks—which have supported decision making in other fields—are incorporated into the risk planning and decision-making framework for coral reef restoration.

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Part VI

Microbiome Biotechnology and Future Outlook

Beyond Restoration: Coral Microbiome Biotechnology

15

Joana F. Couceiro, Rodrigo Costa,
and Tina Keller-Costa

Abstract

Coral-associated microorganisms harbour an impressive and diverse secondary metabolite biosynthesis capacity with novel bioactive compounds being reported every year. The need of the coral holobiont to combat predation, overgrowth, and fouling presumably led to the ability of coral-associated microbes to produce different classes of compounds with a broad spectrum of activities. Although the precise ecological functions of most compounds from coral-associated microbes remain unknown or unproven, the biotechnological applications and prospective benefits of their exploitation are at hand. Bioactivities of pharmaceutical and industrial interest of coral symbiont-derived compounds include antitumoral, antibacterial, antifungal, antifouling, anti-inflammatory, and antidiabetic properties among many others, indicating vast potential for blue biotechnology and blue pharma. Moreover, coral-derived microorganisms often produce enzymes which can be employed in bioindustrial processes or for bioremediation purposes, for instance, in oil spills. This chapter reviews new natural products from coral symbionts reported between the years 2018 and 2022, highlighting the versatility and economic potential of this unique chemical reservoir. More than 385 novel compounds were described from coral-associated microbes in the past 5 years, 75% of them from octocoral (Octocorallia) symbionts. Over 87% of the compounds derive from coral-associated fungi of the Ascomycota phylum while only about 12% come from bacterial associates in the phyla *Actinomycetota*, *Pseudomonadota*, *Bacillota* and *Cyanobacteria*. Terpenes, alkaloids, peptides, and polyketides are the most prominent compound classes, many of which show anticancer, antibacterial, antifungal and antidiabetic activities. Despite the wide compound range described in coral-associated microorganisms, this chapter unveils that most of the lately applied research efforts target only certain microbial groups, such as actinomycetes and fungi, or specific geographical locations (e.g., South China Sea) and coral species. It reveals corals as a warehouses of microbes with bioactive potential similar to what is known from marine sponges. This largely untapped reservoir of novel natural products from coral-associated microbes is yet to be unlocked in future biodiscovery programs. We thus call on the scientific community to expand the scope of their coming research, directing it towards less explored groups such as cold-water corals and non-actinomycete bacterial symbionts. We also suggest a stronger integration of metagenomics libraries, synthetic biology, and heterologous expression approaches to access the chemical space of unculturable coral symbionts, as well as, the inclusion of new cultivation strategies, such as the iChip, for a more comprehensive, polyphasic approach.

Keywords

Secondary metabolism · Natural products · Blue bioeconomy · Bioactivity · Antibiotics · Antifungal Antifouling · Anticancer · Anti-inflammatory Octocorals

15.1 Introduction

Corals (Cnidaria, Anthozoa) are marine, sessile invertebrate animals inhabiting the most varied regions of the globe, from shallow tropical waters to colder and deeper seas (Yoshioka and Yoshioka 1989; Wallace et al. 2001; Pérez et al. 2016). Attached to the ocean floor or hard substrate, they rely on unique symbiotic relationships and elaborate chemical

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defence strategies for survival. The coral holobiont's staggering chemistry assures protection against other organisms such as predators, pathogens and fouling organisms, acting, for example, as feeding deterrents against grazers (Giordano et al. 2017). Coral-derived terpenoids, namely odiferous furanosesquiterpenes protect the Mediterranean octocoral *Maasella edwardsii* by promoting avoidance and memory induced rejection responses in the common predator shrimp *Palaemon elegans* (Giordano et al. 2017). Examples of antibiotic activity include the findings of (Eskander et al. 2018), who reported deleterious effects of soft coral extracts on the hydrophobicity of biofilms formed by *Vibrio harveyi*, an opportunistic coral pathogen. Apart from this, corals experience strong competition for physical space, whether for settlement or expansion of their colonies (Sheppard 1979). Indeed, scleractinian coral extracts have been found to be lethal to several species of coral planulae larvae and even cause adverse effects on newly settled corals, attesting for a fierce competition among distinct species of reef corals (Fearon and Cameron 1997). Along with their importance in defence and competition, corals also rely on chemical compounds to act as signals in various physiological processes and during reproduction. Attraction of sperm to coral colonies, for example, is mediated by chemical substances such as diterpenes (Coll et al. 1995).

Owing to this distinctive lifestyle as well as to their unprecedented species diversity, corals have evolved into one of the most prolific sources of natural products with circa 200 new chemicals of diverse classes being described each year (Carroll et al. 2021, 2022, 2023). Their impressive arsenal of compounds encompasses a large variety of structures, from terpenes, steroids, alkaloids, polyketides, to peptides. These molecules show a wide range of promising applications with several different activities such as antibacterial, anti-inflammatory, anti-fungal, anticancer, or antifouling amongst many others (Raimundo et al. 2018; Sang et al. 2019; Modolon et al. 2020). Some of these compounds became exquisite components of marketable products. Pseudopterosins, a group of diterpene-pentoseglycosides, isolated from a Caribbean octocoral of the species *Antillogorgia elisabethae* (before *Pseudopterogorgia elisabethae*) demonstrate anti-inflammatory and analgesic properties (Look et al. 1986), a discovery that prompted the multinational cosmetics company Estée Lauder to establish a successful line of skin care products with the bioactive ingredient pseudopterosin A (Kijjoa and Sawangwong 2004; Martins et al. 2014).

Most often, however, the commercial development of coral-derived compounds is limited and economically infeasible due to the complexity and low yields of total chemical synthesis of these natural products and the fact that harvesting large amounts of wild corals is unsustainable and has huge repercussions on marine ecosystems (Bruckner 2000). Furthermore, the maintenance of coral farming facilities requires great expenses as a healthy reef requires the presence of many other organisms, such as urchins that graze on infesting algae. Apart from these difficulties, depending on the coral species, growth rate may vary between 0.3 cm to 10 cm per year (Dullo 2005; Brachert et al. 2022), leading to long waiting times for corals to have sufficient size for profitable harvest. However, the current realization that marine host-associated microorganisms produce many of the active compounds found in their host provides unique opportunities for the development of sustainable alternatives for obtention of coral-derived natural products (Moree et al. 2014; Raimundo et al. 2018; Sang et al. 2019; Modolon et al. 2020). Moreover, more than 30 bacteria species associated with corals have been found to produce secondary metabolites (Modolon et al. 2020).

The importance of coral associated microorganisms for reef health is well recognized. One of the best-known examples is the symbiotic relationship with dinoflagellate microalgae of the Symbiodiniaceae family. Symbiodiniaceae are photosynthetic autotrophs which provide their coral hosts with up to 95% of the carbohydrates they produce (Muscatine and Porter 1977). Mydlarz and colleagues demonstrated that *Symbiodinium* sp. is capable of de-novo synthesizing the above described anti-inflammatory pseudopterosins in physiologically significant levels, determining the symbiont origin of these anti-inflammatory compounds (Mydlarz et al. 2003). Indeed, the genomes of Symbiodiniaceae (especially of clades A and C) harbour diverse polyketide synthase and non-ribosomal peptide synthase genes, which likely evolved through numerous events of gene duplication, horizontal gene transfer or recombination, leading to a highly diversified chemical reservoir of secondary metabolites in this taxon (Beedessee et al. 2019). Beyond Symbiodiniaceae and other microalgae, coral microbial symbionts also include fungi, bacteria, archaea and even viruses (Knowlton and Rohwer 2003; Tout et al. 2014; Bourne et al. 2016; Thurber et al. 2017; Keller-Costa et al. 2017). The microbial communities inhabiting corals widely benefit their coral hosts (Peixoto et al. 2017), with corals depending on microbial partners for essential amino acids biosynthesis, vitamin production, sulphur cycling and nitrogen fixation, amongst many others (Shinzato et al. 2011; Neave et al. 2017; Liu et al. 2018a; Robbins et al. 2019; Sweet et al. 2021; Rosado et al. 2023; Doering et al. 2023). Genome sequencing of gammaproteobacterial symbionts such as *Endozoicomonas* or *Halomonas* revealed the presence of nutrient transport systems hypothesised to be required for the maintenance of homeostasis in corals (Meyer et al. 2015; Neave et al. 2017). Coral bacterial symbionts have been found to produce antibiotic compounds that control bacterial populations and inhibit the growth of well-known coral pathogens such as *Vibrio coralliilyticus* (Kvennefors et al. 2012). Indeed, the bioactivities described for coral-symbiont-derived compounds are immense. The following sections will review natural products from coral-associated microorganisms discovered between 2018 and 2022 with diverse activities of interest for biotechnological development.

15.2 Diversity and Provenance of New Natural Products from the Coral Microbiome Discovered Between 2018 and 2022

Here we revisit novel compounds discovered from coral-associated microorganisms between the years 2018 and 2022. For compounds discovered before 2018, we recommend the reviews of Raimundo et al. (2018), Sang et al. (2019) and Modolon et al. (2020). A thorough and systematic review was performed in this study, and the selection of publications included only literature revealing compounds that were completely elucidated in terms of their chemical structures and that were strictly novel. Compounds which were only novel for marine environments but already known from terrestrial habitats were not considered. Additionally, only articles explicitly disclosing the source of the novel compounds as from coral associated microbes were included in the pool of selected publications. This resulted in 111 publications corresponding to 387 novel compounds for the 5-year period considered.

The literature reviewed for this period shows that most of the novel compounds are from octocoral-derived microbes rather than from hexacoral-associated microorganisms. Indeed, more than three fourths of the compounds were obtained from octocoral-associated fungi or bacteria (Fig. 15.1a). Intriguingly, 88% of the compounds described from coral-associated microbes between 2018 and 2022 were obtained from fungi of the Ascomycota phylum while 12% derived from bacteria. The coral-associated bacterial phyla producing novel compounds were *Actinomycetota*, *Pseudomonadota*, *Bacillota* and *Cyanobacteria* (Fig. 15.2b). Surprisingly, no natural products were described from coral-associated *Bacteroidota* which are also common culturable members of the coral microbiome (Keller-Costa et al. 2017; Sweet et al. 2021). At genus level, well-studied taxa such as *Penicillium*, *Aspergillus* and *Streptomyces* clearly dominate natural product production in the cultured coral microbiome. The bacterial genera *Labrenzia*, *Microbulbifer*, *Micrococcus* and *Kocuria* are also notable contributors (Fig. 15.1c, d) and, although they are less known for their natural product production capacities, they are frequently isolated from corals (Keller-Costa et al. 2017; Sweet et al. 2021). Notably, bioactive compound producing fungi, mainly *Aspergillus* and *Penicillium,* but also *Xylaria*, *Simplicillium*, *Trichoderma*, *Stachybotrys* and *Talaromyces*, amongst others, were mostly obtained from octocorals, while natural product producing *Parengyodontium* and *Acremonium* were mostly described in hexacorals (Fig. 15.1d). It remains unclear, however, whether this trend is the result of sampling or cultivation biases, or a reflection of true fungal community patterns.

The coral-associated microbes were found to produce mainly alkaloids, polyketides, peptides, and terpenes or terpenoids and their derivatives. The alkaloids were generally associated with antitumoral or anticancer activities while terpenes, terpenoids, peptides and polyketides showed a broad spectrum of bioactivities. Notably, a significant number of novel compounds did not have bioactivities associated and characterized. Moreover, the types of bioactivities reported are highly dependent on the testing efforts of the research laboratories and their capacities to perform specific and broad range activity screenings. Large biases presumably exist, since certain activities, such as e.g., antibacterial, and antitumor activities are more commonly studied, while many other activities, e.g., antidiabetic, antineuropathic, and even antifouling, seem to be less frequently tested for.

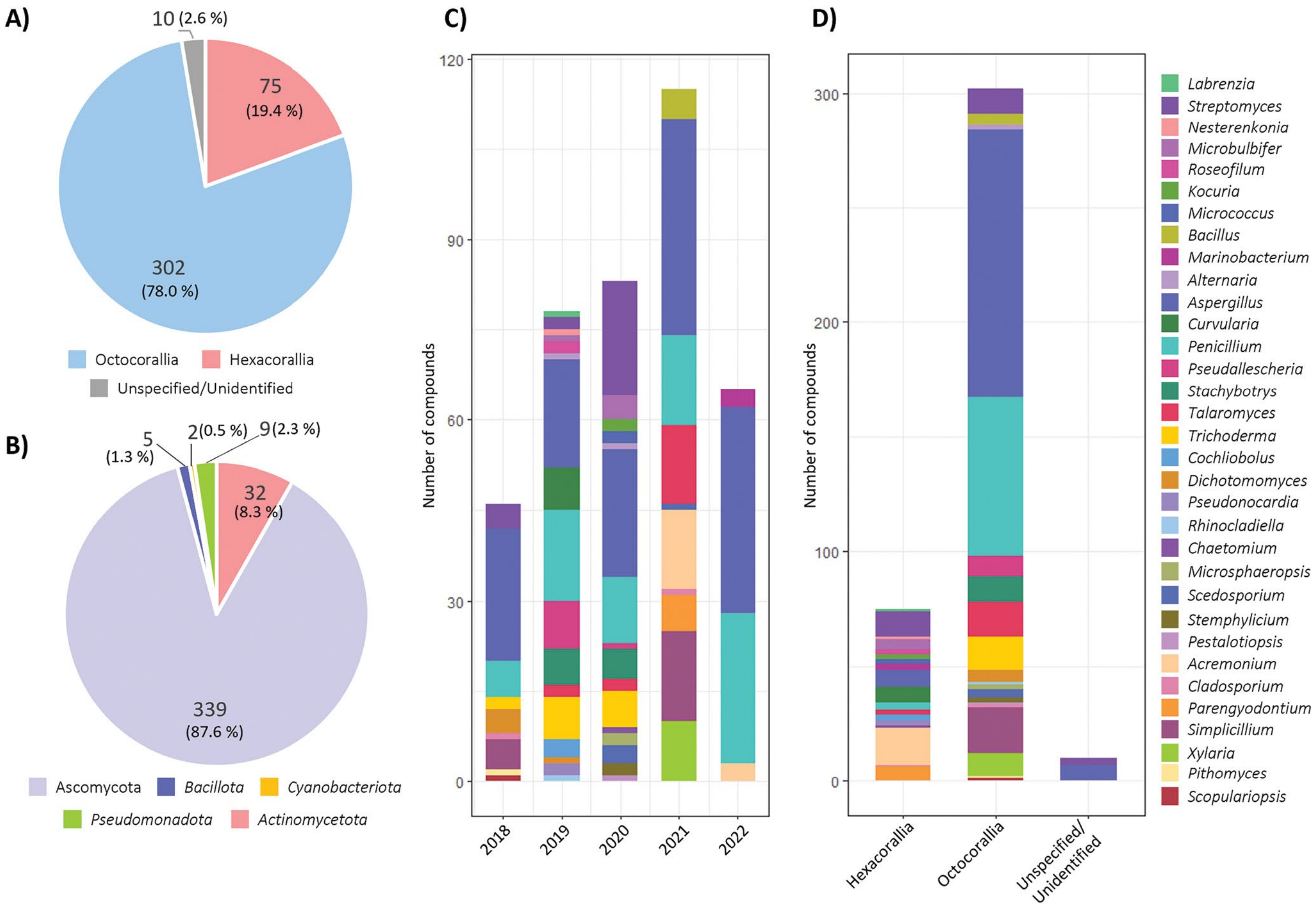


Fig. 15.1 Provenance of new natural products from coral-associated microbes reported between 2018 and 2022, considering (**a**) coral subclass, (**b**) microbial phylum, (**c**) genus of the natural product producing microbial associate by year of report and (**d**) genus of the natural product producing microbial associate by coral subclass. Note that the genus legend on the right is the same for panels (**c**) and (**d**). Numbers in the figure indicate the number and percentage of compounds in each category

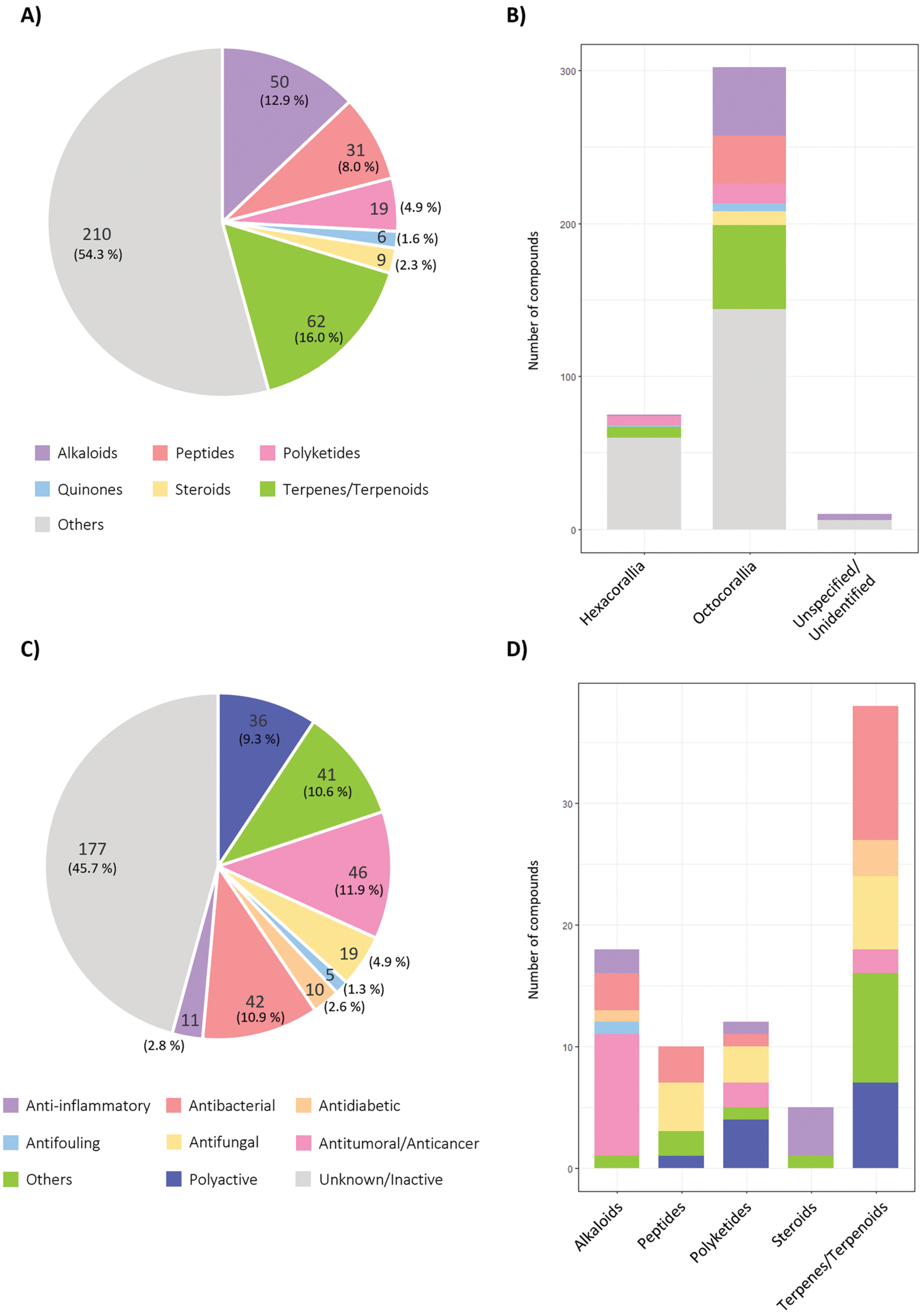


Fig. 15.2 Provenance of new natural products from coral-associated microbes reported between 2018 and 2022 considering (**a**) compound classes and (**b**) compound classes per coral subclass, (**c**) compound activity and (**d**) compound activity by compound class. Note that the colour code of panel (**b**) is the same as panel (**a**). The colour code of panel (**d**) is the same as panel (**c**) with the exception that compounds with no or unknown activity (grey) are not shown in (**d**) to facilitate visualisation. Numbers in the figure indicate the number and percentage of compounds in each category

15.3 Antibacterial Activity: In Search for New Antibiotics

Antibiotic resistance is one of the main threats for global health, food security and development (Nji et al. 2021; Samtiya et al. 2022). There were 1.27 million deaths attributable to bacterial antimicrobial resistance in 2019 alone (Murray et al. 2022). The World Health Organization (WHO) has published a list of the most concerning antibiotic-resistant bacteria, for which novel, effective antibiotics are necessary. This includes critical priority species such as carbapenem-resistant and extended spectrum β-lactamase (ESBL) producing *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae* bacteria and high priority organisms such as methicillin-resistant *Staphylococcus aureus,* clarithromycin-resistant *Helicobacter pylori,* and multidrug-resistant *Neisseria gonorrhoeae*, *Campylobacter*, *and Salmonella* spp. (World Health Organization 2017). The origins of bacterial resistance to antibiotics are varied. While some bacteria display natural resistance, either always expressed or induced by adaptation to the continued presence of the antibiotic, many strains have acquired resistance from their surrounding environment, through horizontal gene transfer or mutations on their genomes (Reygaert 2018). The mechanisms which bacteria employ to evade the effects of these compounds are extremely versatile, from inactivating or constraining the uptake of a bioactive molecule, modifying the cellular target for the drug or having active drug efflux pumps (Reygaert 2018).

The recent isolation of the Sodariomycetes fungus *Simplicillium* sp. SCSIO 41513 from an octocoral collected in the South China Sea yielded eleven new fusidane-type nortriterpenoids (Cheng et al. 2021). These 11 simplifusidic acids had different chemical substituents and differing antibacterial potency related to their structure. Simplifusidic acid I (Fig. 15.3 and Table 15.1) showed potent antibacterial activity against *Staphylococcus aureus*, with minimum inhibitory concentration (MIC) values of 0.078 μg/mL, three times lower than the activity of the commonly used antibiotic fusidic acid (0.25 μg/mL) (Fernandes 2016). Another example of a recently discovered, potent antibacterial compound is isotirandamycin B (Fig. 15.3 and Table 15.1) produced by the *Actinomycetales* bacterium *Streptomyces* sp. SCSIO 41399 isolated from the stony coral *Porites lutea* (Cong et al. 2019). This compound displays potent activity against *Streptococcus agalactiae*, which has been recognized as one the most prevalent causes of neonatal life-threatening bacterial infections (Schuchat 1999).

The fungus *Aspergillus versicolor* CHNSCLM-0063 collected from the gorgonian coral *Rumphella aggregata*, produced three new cycloheptapetides, asperversiamides A-C (Table 15.1) which displayed potent antibacterial activity against *Mycobacterium marinum* (Hou et al. 2019b). This pathogen causes tuberculosis-like illness in both saltwater and freshwater fish species. It is not only a serious threat to aquaculture but also a human health concern because of its close relatedness with *Mycobacterium tuberculosis* (Aubry et al. 2017). Aquaculture is a growing industry with tremendous importance for the global economy. In 2020, alone, 87.5 million tonnes of fish were produced in aquaculture, and the prediction for 2030 is of 202 million tonnes, according to the Food and Agriculture Organization of the United Nations (FAO) (Food and Agriculture Organization 2022). The sector is facing increasing antimicrobial resistance among aquaculture pathogens with a very high risk for infections to cross from animals to humans (Sanches-Fernandes et al. 2022) that needs to be urgently addressed with novel antimicrobials and other innovative products and solutions.

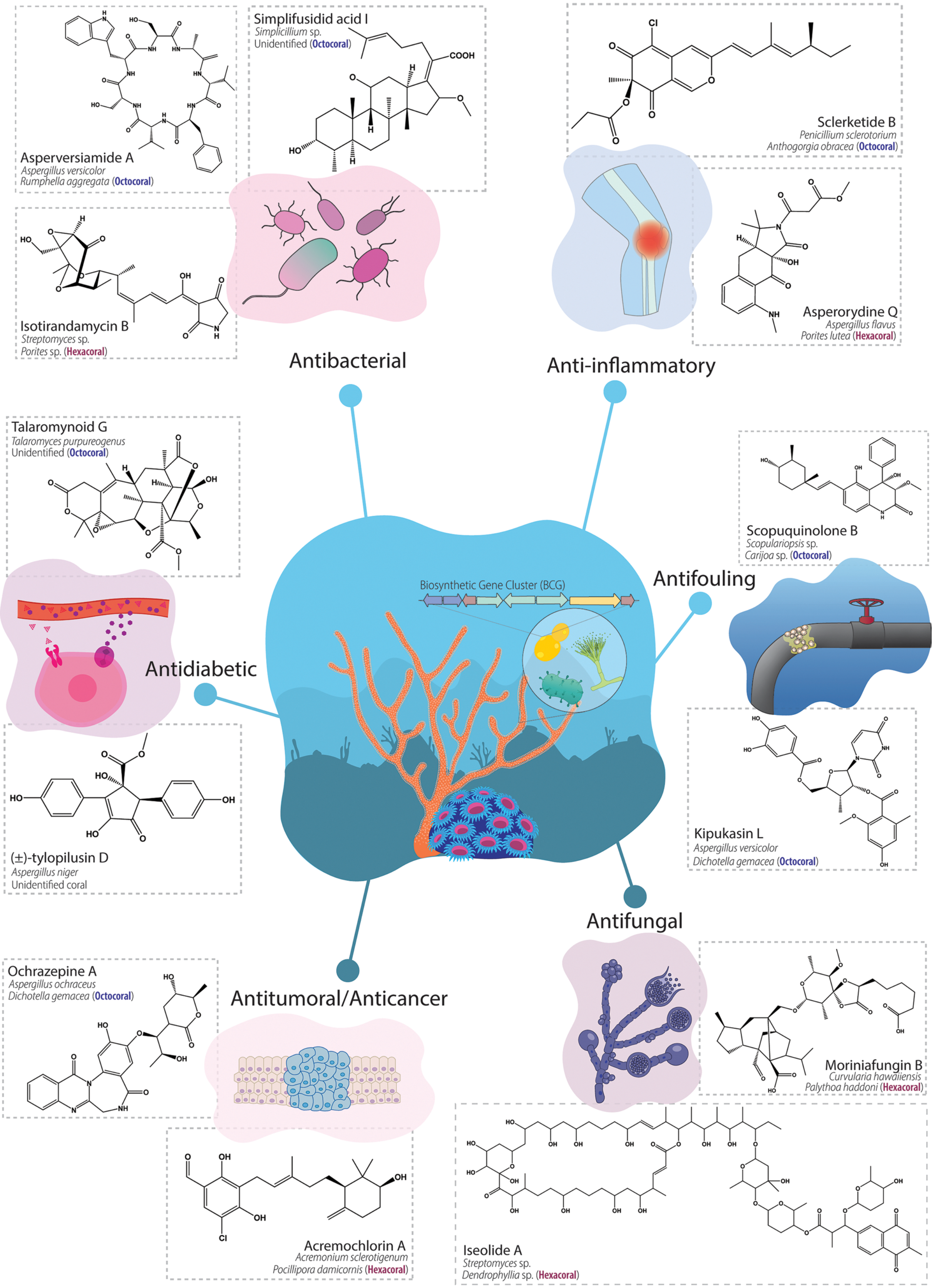


Fig. 15.3 Examples of the diverse chemical structures and common activities of compounds produced by coral-associated microorganisms

Table 15.1 Summary of new bioactive natural products described in this chapter. The compounds were selected from the collection of 387 novel compound reports from coral-associated microorganisms in the period of 2018 to 2022

Compound name	Compound class	Microbial producer	Coral host	Activity	Reference
Simplifusidic acid I	Terpenes/ Terpenoids	*Simplicillium* sp.	Unidentified octocoral	**Antibacterial** Against *Staphylococcus aureus*	Cheng et al. (2021)
Isotirandamycin B	Others	*Streptomyces* sp.	*Porites* sp. (Hexacoral)	**Antibacterial** Against *Streptococcus agalactiae*	Cong et al. (2019)
Asperversiamides A-C	Peptides	*Aspergillus versicolor*	*Rumphella aggregata* (Octocoral)	**Antibacterial** Against *Mycobacterium marinum*	Hou et al. (2019b)
(±)-Tylopilusin D	Diphenol derivative	*Aspergillus Niger*	Unidentified/ Unspecified	**Antidiabetic** Tyrosine phosphatase (PTP) inhibitor	Kim et al. (2020)
Talaromynoids G-I	Terpenes/ Terpenoids	*Talaromyces purpureogenus*	Unidentified octocoral	**Antidiabetic** Triglyceride accumulation reduction in adipocytes	Huang et al. (2021)
Versilactone G	Butenolide derivative	*Aspergillus terreus*	*Sarcophyton subviride* (Octocoral)	**Antidiabetic|anti-inflammatory (Polyactive)** α-Glucosidase inhibitorynoid	Wu et al. (2020)
Scopuquinolone B	Alkaloid	*Scopulariopsis* sp.	*Carijoa* sp. (Octocoral)	**Antifouling** Against *Amphibalanus amphitrite* larval settlement	Mou et al. (2018)
Kipukasin L	Nucleoside derivative	*Aspergillus versicolor*	*Dichotella gemmacea* (Octocoral)	**Antifouling** Against *Bugula neritina* larval settlement	Wu et al. (2020)
Carneusin B	Others	*Aspergillus carneus*	*Anthogorgia* sp. (Octocoral)	**Antifouling** Against *vibrio rotiferianus* and *Alteromonas macleodii*	Xu et al. (2023)
Moriniafungines B-G	Terpenes/ Terpenoids	*Curvularia hawaiiensis*	*Palythoa haddoni* (Hexacoral)	**Antifungal** Against *Candida albicans*	Zhang et al. (2019)
Iseolides A-C	Polyketides	*Streptomyces* sp.	*Dendrophyllia* sp. (Hexacoral)	**Antifungal** Against *Candida albicans*, *Trichophyton rubrum* and *Glomerella cingulate*	Zhang et al. (2020)
Sclerketide B	Polyketide	*Penicillium sclerotorium*	*Anthogorgia obracea* (Octocoral)	**Anti-inflammatory** NO production inhibitor	Liu et al. (2019b)
Asperorydine Q	Alkaloid	*Aspergillus flavus*	*Porites lutea* (Hexacoral)	**Anti-inflammatory** NF-κB pathway activation inhibitor	Wang et al. (2022)
Ochrazepines A-D	Circumdatin-aspyrone conjugates	*Aspergillus ochraceus*	*Dichotella gemmacea* (Octocoral)	**Antitumoral/anticancer** Against several cell lines (*see in text*)	Fan et al. (2019)
Cochliobopyrones A-B	α-Pyrones	*Cochliobolus lunatus*	Unidentified hexacoral	**Antitumoral/anticancer** Type I topoisomerase inhibitor	Wu et al. (2019)
Acremochlorins A-M	Ascochlorin	*Acremonium sclerotigenum*	*Pocillipora damicornis* (Hexacoral)	**Antitumoral/anticancer** Against triple-negative breast cancer (TNBC) cell lines, MDA-MB-231/−468	Luo et al. (2021)
Chrysogeamides A-B	Peptides	*Penicillium chrysogenum*	*Carijoa* sp. (Octocoral)	**Angiogenic**	Hou et al. (2019a)

(continued)

Table 15.1 (continued)

Compound name	Compound class	Microbial producer	Coral host	Activity	Reference
Sesquiterpene derivative (sum formula $C_{15}H_{23}ClO_5$)	Terpenes/ Terpenoids	*Pseudallescheria boydii*	*Sclerophytum sandensis* (Octocoral)	**Osteoclastogenic**	Liu et al. (2019a)
Phenylcandilide A-B	p-Terphenyl derivative	*Aspergillus candidus*	*Junceela fragilis* (Octocoral)	**Neuromodulatory** Effects on spontaneous Ca^{2+} oscillations (SCOs)	Peng et al. (2021)
Asperindole G	Alkaloid	*Aspergillus candidus*	*Junceela fragilis* (Octocoral)	**Neuromodulatory** Effects on spontaneous Ca^{2+} oscillations (SCOs)	Peng et al. (2021)
16,17-Dihydroxy-deoxydihydroisoaustamide, 16β,17α-Dihydroxy-deoxydihydroisoaustamide, 16α,17α-Dihydroxy-deoxydihydroisoaustamide	Deoxyisoaustamide derivative	*Penicillium dimorphosporum*	Unidentified Octocoral	**Neuroprotective**	Zhuravleva et al. (2021)
Harzianelactone A-B, Harzianone A-D and Harziane	Terpenes/ Terpenoids	*Trichoderma harzianum*	Unidentified Octocoral	**Phytotoxic**	Zhao et al. (2019)

15.4 Antifungal Activity

Ubiquitous to all environments on earth, fungi are not only crucial organic matter decomposers and consummate nutrient recyclers, but some are also an underestimated threat to human health. Currently, the impact of fungal pathogens is profound, with over 150 million people having serious fungal infections that translate into major impacts in their daily life or constitute fatal infections (Bongomin et al. 2017). The most common fungal infections include nail infections, vaginal candidiasis, ringworm (fungal skin infections) and mouth, throat, or oesophagus infections with *Candida* spp. Moreover, infections with emerging, multi-drug resistant *Candida auris* are a concern for hospitals and health care infrastructures (Egger et al. 2022). Additionally, fungi have, indeed, an extraordinary ability to adapt and respond to environmental pressures, easily acquiring resistance to existing antifungal agents. The misuse of antifungal drugs is causing fungal strains to develop resistance to currently marketed antifungal drug classes which include a small diversity of structures, mostly azoles, polyenes, allylamines and echinocandins (Chen and Sorrell 2007). Coral-reef ecosystems do not escape the threat of fungal pathogens with notorious examples such as aspergillosis outbreaks in sea fans, causing massive mortalities in different octocoral species (Roik et al. 2022). The underlying principle that the coral microbiome is of enormous relevance for the defence against coral pathogens, which include fungi, drives the search for much needed, novel antifungal agents from marine settings. The Dothideomycetes mold *Curvularia hawaiiensis* TA26-15, isolated from the zoanthid *Palythoa haddoni*, produces tetracyclic diterpene glycosides, moriniafungines B-G (Fig. 15.3 and Table 15.1) which display antifungal activity against *Candida albicans*. Moriniafungine E exhibits particularly strong activity towards *C. albicans* with a reported MIC value of 2.9 nmol/mL. These compounds possess a quite unusual structure consisting of a sordarose (sugar moiety) with a spiro 1,3-dioxolan-4-one ring which is rare in nature and unprecedented in marine organisms (Zhang et al. 2019).

The impact of fungal pathogens in society goes far beyond their ability to infect humans, as they are able to destroy a third of the annual food crop production, threatening food supply chains (Fisher et al. 2012; Almeida et al. 2019). Three new glycosylated macrolides, called iseolides A-C (Fig. 15.3 and Table 15.1), were recently isolated from the culture broth of *Streptomyces* sp. DC4-5, an *Actinomycetes* bacterium derived from the hexacoral *Dendrophyllia* sp. (Zhang et al. 2020). These compounds showed remarkable activities not only against the human pathogens *C. albicans* and *Trichophyton rubrum*, but also towards *Glomerella cingulate* (Zhang et al. 2020), a phytopathogen which causes anthracnose and fruit rotting disease in manifold economically relevant crop species, including cereals, legumes, vegetables and fruits such as strawberry, peach and mango (Rittenburg and Henrix Jr 1983; Howard and Albregts 1984; Freeman et al. 1998; Xie et al. 2010; Onyeani et al. 2012).

15.5 Antidiabetic Activity

There are, currently, around 537 million people living with a form of diabetes worldwide, according to data from 2021 (International Diabetes Federation 2021). Diabetes constitutes the major cause of blindness, kidney failure, heart attack and strokes (World Health Organization 2023) and occurs when the pancreas is no longer able to produce insulin in sufficient amounts or when the body is incapable of utilising it normally (World Health Organization 1999). This rapidly growing problem prompted the search for natural products effective in mitigating the symptoms and consequences of this chronic disease. The hypoglycaemic effects of traditional medicinal plants are already widely studied (Tran et al. 2020). Coral-derived microbes are now arising as a novel, promising source of antidiabetic compounds.

Aspergillus niger SF-5929, isolated from an Antarctic coral (species unknown) produces (±)-tylopilusin D (Fig. 15.3 and Table 15.1), a diphenolic metabolite that displays potent inhibitory effects on the enzyme tyrosine phosphatase PTP1B (Kim et al. 2020). PTP1B has been recognized as a potential target for the treatment of diabetes as it is usually involved in the regulation of insulin sensitivity (Ma et al. 2011). 3,5-Dimethylorsellinic acid (DMOA)-derived meroterpenoids, talaromynoids G-I (Fig. 15.3 and Table 15.1), isolated from another octocoral-associated fungus, *Talaromyces purpureogenus* SCSIO 41517, also revealed antidiabetic potential by lowering triglyceride accumulation in 3T3-L1 adipocytes (Huang et al. 2021). Although DMOA-derived meroterpenoids are known to possess various promising bioactivities, the highly oxygenated talaromynoids G-I had unprecedented polycyclic systems, never reported before, paving the way to new chemical structures of interest to antidiabetics drug research (Huang et al. 2021).

Additionally, the treatment of type 2 diabetes has long relied on α-glucosidase inhibitors, as a substitution for the widely prescribed type 2 diabetes drug metformin, and their efficacy has been proven (Joshi et al. 2015). Alpha-glucosidase inhibitors have the ability to delay glucose absorption in the small intestine, thus controlling hyperglycaemia (Hossain et al. 2020). Recently, a new polyactive butenolide derivative, versicolactone G (Table 15.1), was obtained from an *Aspergillus terreus* strain isolated from the deep-sea octocoral *Sarcophyton subviride*. Among the several activities exhibited by this compound, it showed remarkable α-glucosidase inhibitory activity, with an IC_{50} value below that of the currently marketed anti-diabetic drug acarbose (Liu et al. 2018b).

15.6 Anti-Inflammatory Activity

Inflammation is a biological response that works as a defence mechanism which can be activated by several different triggers, such as pathogens, damaged cells, and toxins. The objective of this response is to diminish the detrimental stimuli that activated it. However, an uncontrolled and dysregulated response can have harmful effects in organisms (Medzhitov 2008; Takeuchi and Akira 2010; Chen et al. 2018). The most commonly activated signalling pathways include the nuclear factor-κB (NF-κB), the mitogen-activated protein kinase (MAPK) and the Janus kinase/signal transducer and activator of transcription pathway (JAK-STAT) which act by recognising stimuli (pattern recognition receptors) and transducing signals to cell nuclei for differential gene expression (Ahmed et al. 2015). Anti-inflammatory compounds have long been a popular target for coral microbiome-derived activities since the isolation success of the pseudopterosins, diterpene glycosides with anti-inflammatory and analgesic properties from *Antillogorgia elisabethae* (before *Pseudopterogorgia elisabethae*) in 1986 (Look et al. 1986) and the discovery that the endosymbiont *Symbiodinium* sp. type B1 (now classified as *Breviolum*) synthesizes the pseudopterosins A to D (Mydlarz et al. 2003). Pseudopterosin A has undergone Phase II human clinical trials (Ruiz-Torres et al. 2017) and is the ingredient of skin creams (Kijjoa and Sawangwong 2004; Martins et al. 2014).

Penicillium sclerotorium isolated from the octocoral *Anthogorgia obracea* produces several novel polyketides, one of which, sclerketide B (Table 15.1), was demonstrated to possess potent anti-inflammatory activity by inhibiting the production of nitric oxide (NO) in lipopolysaccharide (LPS)-induced macrophage cells (Liu et al. 2019b). Another fungal strain, *Aspergillus flavus*, isolated from the hexacoral *Porites lutea* produces a new alkaloid, asperorydine Q (Fig. 15.3 and Table 15.1), able to interfere with the NF-κB pathway (see above) by inhibiting the activity of LPS-induced NF-κB activation (Wang et al. 2022).

15.7 Antitumoral/Anticancer Activity

Cancer is currently a leading cause of death worldwide, claiming around ten million lives yearly, with the most prevalent cancers being breast, lung, colon, and rectum cancer (Ferlay et al. 2020). The transformation of normal cells into pre-cancerous cells and eventually into malignant tumours is usually propelled by the interaction between genetic factors and external agents such as UV radiation, carcinogenic compounds (e.g. asbestos, arsenic), viral (e.g. hepatitis, human papillomavirus), bacterial (e.g. *Helicobacter pylori*) or parasite infections and/or abuse of tobacco, alcohol and other drugs (Borek 1993; Stein and Colditz 2004; Szymonowicz and Chen 2020; Dalsgaard et al. 2021). The search for natural products with anticancer or antitumoral activity has been established for long with about 25% of approved compounds being natural products or derivatives thereof (Newman and Cragg 2020), including several success stories of marketed anticancer drugs from marine invertebrates, e.g., cytarabin and eribulin mesylate (trade name Halaven®) from marine sponges, trabectedin (trade name Yondelis®) from a tunicate and brentuximab vedotin (trade name Adcetris®) from a mollusc (Ruiz-Torres et al. 2017).

In 2019, four novel compounds were isolated from *Aspergillus ochraceus* associated with the octocoral *Dicothella gemmacea*: ochrazepines A-D (circumdatin-aspyrone conjugates) (Table 15.1). These compounds showed very promising activities for cancer treatment:

Ochrazepine A (Fig. 15.3) showed strong activities against 10 different cancer cell lines; Ochrazepines B and D had selective activity against a human gliosblastoma cell line (U251) and ochrazepine C had activity against three human cancer cell lines, i.e., A673 (rhabdomyoma), U87 (glioblastoma) and Hep3B (liver cancer). Furthermore, these compounds showed low cytotoxicity against healthy human cell lines (Fan et al. 2019). In another study, the fungus *Cochliobolus lunatus* TA26-46, isolated from an unidentified coral, was co-incubated with DNA methyltransferase inhibitors resulting in distinct metabolite profiles. This epigenetic manipulation yielded two new α-pyrones, cochliobopyrones A and B (Table 15.1) which exhibited Type I topoisomerase (Topo I) inhibitory effects (Wu et al. 2019). Additionally, 13 new ascochlorin derivatives, acremochlorins A-M (Fig. 15.3 and Table 15.1) were isolated from the fungus *Acremonium sclerotigenum*, which displayed inhibition of human dihydroorotate dehydrogenase (hDHODH), a common tumour target for the treatment of cancer, as well as pronounced inhibitory activity against triple-negative breast cancer (TNBC) cell lines, MDA-MB-231/−468 (Luo et al. 2021).

15.8 Antifouling Activity: Eco-Friendly Solutions for the Maritime Industry

The adhesion and accumulation of microorganisms, algae, seaweeds, and/or small animals on marine infrastructures such as submarine gas or oil pipelines, offshore drilling rigs, turbines or on the hulls of ships is a major threat for maritime industries as well as for marine ecosystems. The problems arising from biofouling and biocorrosion in, for instance, oil or gas submarine pipes cause expensive and irreversible losses. The 15-year cumulative, predicted spending on biofouling mitigation for a standard navy vessel surpasses 40 million US dollars (Schultz et al. 2011), a major burden for industries dependant on maritime transport. The costs associated with biofouling for ships are mainly due to an increase in the amount of fuel needed to operate it (Schultz et al. 2011). The increased drag created by the presence of fouling organisms leads the increased demand for fuel, and, consequently, a larger amount of pollutant gases is released to the atmosphere. Additionally, marine ecosystems also face enhanced pressures with the possible introduction of non-indigenous species as ships act as vectors for transference of biofouling species from their native habitats to new ones (Luoma et al. 2021). Throughout history, several antifouling compounds have been employed in coatings, but the toxicity and harmful effects of many of these compounds on the environment (Amara et al. 2018) have pushed policy makers to regulate the use of such coatings. Hence the necessity to search for alternative, eco-friendly substances capable of inhibiting or diminishing fouling while not harming marine wildlife. Marine symbionts associated with sessile, invertebrate animals are a unique source of antifouling compounds, following the hypothesis that these microorganisms developed powerful chemical defence strategies to protect their animal host (Satheesh et al. 2016).

The fungus *Scopulariopsis* sp. isolated from the octocoral *Carijoa* sp. produces the alkaloid scopuquinolone B (Fig. 15.3 and Table 15.1) with antifouling activity against the barnacle *Amphibalanus amphitrite*, inhibiting larval settlement (Mou et al. 2018). *Aspergillus versicolor* XS-20090066 isolated from octocoral *Dichotella gemmacea*, produces kipukasin L (Fig. 15.3 and Table 15.1), a nucleoside derivative able to inhibit the settlement of larvae from the bryozoan *Bugula neritina* (Wu et al. 2020). Besides sessile macroorganisms, microfoulers play a pivotal role in the corrosion of maritime structures and are often the initiators of the process, creating biofilms which attract and promote the settlement of larger organisms (Liu et al. 2022). Carneusin B (Table 15.1), obtained from the culture broth of *Aspergillus carneus* GXIMD 00519 isolated from the octocoral *Anthogorgia* sp. shows anti-microfouling effects against the biofouling-promoting marine bacteria *Vibrio rotiferianus* and *Alteromonas macleodii* (Xu et al. 2023).

15.9 Other Activities of Biomedical and Biotechnological Interest

Although the previously described activities represent most of the currently known pharmaceutical and biotechnological potential, coral-associated microorganisms are versatile secondary metabolite producers with diversified pathways and their bioactivity spectrum extends far beyond the above described activities (Raimundo et al. 2018; Sang et al. 2019).

The restoration and repair of tissues and organs is essential for eumetazoan animals and, in vertebrates, relies heavily on the growth of blood vessels, a process known as angiogenesis (Carmeliet 2005). *Penicillium chrysogenum* CHNSCLM-0003 obtained from the octocoral *Carijoa* sp. produces two novel peptides, chrysogeamides A and B (Table 15.1), which were tested for angiogenic activity and showed promising results in zebrafish assays while simultaneously not having toxic effects in embryonic zebrafish (Hou et al. 2019a). Another important process for the human body is the maintenance of a balance between osteoclasts (for bone resorption) and osteoblasts (for bone formation) as this is very dynamic tissue, suffering continuous remodelling (Kim 2022). An impairment or decline in osteoclastogenesis may result in osteosclerosis or bone deficiency. The fungus *Pseudallescheria boydii* isolated from the octocoral *Sclerophytum sandense* (before *Sinularia sandensis*) produces a halogenated sesquiterpene derivative (sum formula

$C_{15}H_{23}ClO_5$) (Table 15.1) that shows osteoclastogenic activity (Liu et al. 2019a). Phenylcandilide A and B (p-terphenyl derivative) and asperindole G (alkaloid) (Table 15.1) are new metabolites isolated from an *Aspergillus candidus* strain associated with the octocoral *Junceella fragilis*, which display neuromodulatory effects (Peng et al. 2021). The authors analysed the compounds' effects on spontaneous Ca^{2+} oscillations (SCOs) which play an important role in mediating neuronal development (Cao et al. 2014). Moreover, *Penicillium dimorphosporum* isolated from an unidentified octocoral produced three new deoxyisoaustamide derivatives (Table 15.1) which showed neuroprotective activity (Zhuravleva et al. 2021). All these just mentioned activities are excellent examples of the understudied and varied putative biomedical applications of natural products from the coral microbiome.

Beyond biomedicine, compounds and enzymes derived from coral-associated bacteria and fungi find other biotechnological applications, not only in the blue bioeconomy sector, but also in distant industries such as agriculture. From the octocoral-derived fungus *Trichoderma harzianum* XS-20090075, authors were able to isolate potent phytotoxic harziane diterpenes (Table 15.1). These diterpenes inhibited seedling growth of amaranth and lettuce hinting at a possible application as natural herbicides in weed control (Zhao et al. 2019).

Vilela et al. (2021) screened a collection of 37 bacteria isolated from the hexacoral species *Mussismilia braziliensis*, *Millepora alcicornis*, and *Porites astreoides* for their production of commercially important enzymes. The isolates displayed a wide range of biocatalytic capacities of biotechnological interest, encompassing lipase, caseinase, keratinase, amylase, gelatinase, chitinase and cellulase activity. In 2023, scientists reported significant antioxidant activity and UVA sunblock effects of carotenoids derived from a *Virgibacillus* strain associated with the octocoral *Sinularia* sp. (Kusmita et al. 2023). Some microbial associates of hexacorals also possess oil degradation capacities which can be useful in bioremediation campaigns during oil spills on reef sites (Villela et al. 2019). The strains *Cobetia* sp. LC_6, *Halopseudomonas aestusnigri* LC_3, *Shewanella algae* LC_4, and *Brucella intermedia* LC_5 were successfully used in a microbial bioremediation consortium for coral which showed higher crude oil degradation capacities compared to control samples and reduced toxic effects on the fire coral *Millepora alcicornis* (Silva et al. 2021). These strains host genes for naphthalene, toluene and quinate degradation as well as for biosurfactant and rhamnolipid biosynthesis (Villela et al. 2023). The plethora of coral-microbiome derived bioactivities highlights their vast potential and prospective benefits for a wide range of industries.

In addition to their potential to mitigate toxic compounds in the surrounding environment, coral-associated microbes also provide several other beneficial roles that can be used for restoration and rehabilitation efforts, as summarized by (Peixoto et al. 2022; Voolstra et al. 2024) and further discussed in Chap. 13.

15.10 Future Directions

15.10.1 Overcoming Cultivation Bias and Regional Limitations in Coral Microbiome Natural Product Discovery

There is an inarguable potential for blue growth in coral associated microorganisms. The phylogenetic diversity of these early-derived animals and, consequently, the plethora of host-microbe and microbe-microbe interactions resulting from this diversity leads to a remarkable collection of compounds of the most varied structures and bioactivities, as described above.

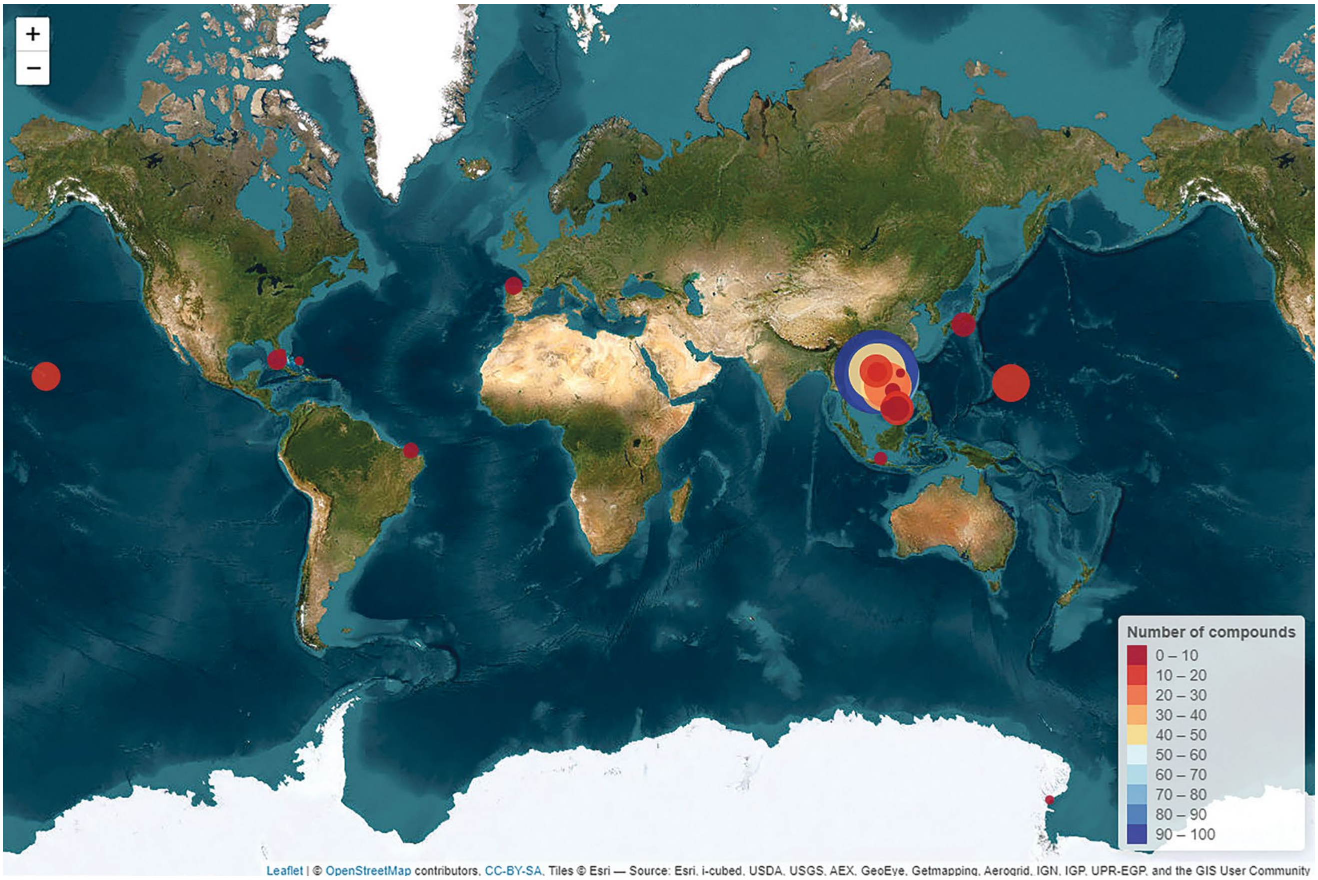
This review revealed, however, a significant difference in the number of novel compounds described from hexacoral *versus* octocoral-derived micro organisms, the latter seeming to be much more prolific producers. From an ecological perspective, most octocorals lack the massive stony skeleton that is characteristic of hexacorals and confers physical resistance. Likely, for octocorals, chemical defence is pivotal to deter predators and avoid overgrowth and fouling. Nevertheless, the selection of coral species for sampling might also be biased, as octocorals are often easier to process. Thus, sampling frequency could, at least partially, be responsible for the larger number of novel compounds described in Octocorallia compared with Hexacorallia.

Another intriguing finding is the dominance of coral fungi-derived over bacteria-derived compounds. To obtain a compound in a quantity that allows complete elucidation of the underlying molecular structure and bioactivity assays, it is commonly necessary to culture the microorganism in the laboratory. This also deters the harvesting of excess tissue biomass from organisms that are threatened. Thus, differences in the cultivability of microbial taxa will lead to a differential representation of their secondary metabolites. This review unveiled the fungal genera *Penicillium* and *Aspergillus* as the most common sources of new natural products from coral-associated microorganisms. Unarguably, both genera are widely known for their great ability to synthesize diverse secondary metabolites and their natural production capacity is well explored and understood (Bills and Gloer 2016). Still, cultivation bias is likely impacting this outcome since both taxa are generally very amenable to cultivation and optimized culture media and conditions exist for them. Cultivation of coral bacteria is frequently a more challenging task and requires fine-tuning of conditions to accommodate rarer and slow-growing taxa (Fig. 15.5). Host-associated bacteria often rely on their symbiotic relationship in an obligate manner and standard laboratory culturing conditions rarely meet their growth requirements. A study on the temperate octocoral *Eunicella labiata* compared the cultivation-dependent *versus* independent bacterial fraction of this species, concluding that high representativity of the prokaryotic community may be obtained by adjusting growth conditions, i.e., lowering incubation temperatures, increasing incubation time and using diluted marine culture media (Keller-Costa et al. 2017). Likewise, a meta-analysis on 3055 bacterial isolates from 84 coral species around the globe, and isolated on 14 cultivation media, counted 138 formally described and 12 putatively novel cultured genera across the *Pseudomonadota*, *Bacillota*, *Bacteroidota*, and *Actinobacteriota* phyla, indicating that culture medium diversification is a sound way of enhancing the genus-level diversity of coral bacterial isolates (Sweet et al. 2021). Further strategies to enhance the cultivability of symbiotic microorganisms include the supplementation of culture media with host tissue extracts, as described for marine sponges, with the aim of providing essential host-derived cues and growth factors that obligate symbionts may rely on (Sipkema et al. 2011). A more complex approach is *in situ* cultivation which minimizes differences between the original environment and the cultivation environment by inserting diffusion chambers with membranes that mediate the exchange of growth factors and nutrients into the host organisms' bodies in their natural environment (Kaeberlein et al. 2002; Steinert et al. 2014; Jung et al. 2021; Steinert et al. 2014; Jung et al. 2021). The iChip, composed of several hundred miniature diffusion chambers, is among the latest technological advances, moving this field into culturomics, i.e., the high-throughput cultivation of microorganisms (Nichols et al. 2010). Indeed, such *in situ* approaches could increase the cultivability of coral-associated microbes up to 570% (Modolon et al. 2023) and promote the growth of previously uncultured bacteria. Other innovative, targeted approaches may allow to isolate other, yet uncultured microorganisms previously identified by cultivation independent methods such as metagenomic binning or single cell genomics. Genome-based investigation of bacterial metabolism allows the design of culture media that favour the growth of certain microorganisms. Therefore, the German Collection of Microorganisms and Cell Cultures (DSMZ) developed MediaDive, the world's largest curated culture media database with recipes and compositions of more than three thousand media (Koblitz et al. 2023). A new feature of MediaDive incorporates prediction based-artificial intelligence to custom design culture media that meet the nutritional requirements of yet uncultured strains for which metagenome-assembled genomes (MAGs), or single-amplified genomes (SAGs) exist. Finally, enrichment cultures allow the isolation of novel marine microbes not able to grow alone but instead depend on other strains e.g., for nutrient exchange or toxic compound removal (Lewis et al. 2010; Wiegand et al. 2019). The marine photosynthetic cyanobacterium *Prochlorococcus*, for example, inhabits illuminated layers of the ocean where reactive oxygen species are abundant, but its genome does not encode for catalase and other protective mechanisms. In turn, this species' protection against oxidation is provided by concomitant heterotrophs able to fulfil this role (Morris et al. 2011; Stewart 2012). All above-described methods are valuable tools that should be tried in future bioprospecting-oriented cultivation attempts to widen the functional diversity of coral-associated bacteria.

Our survey also found sampling efforts to be considerably skewed. Publications reporting novel compounds from coral-associated microbes focus mainly on the South China Sea as a coral sampling site (Fig. 15.4). Although this area is a hot spot for tropical and shallow water coral diversity (Huang et al. 2015), it does not represent the global distribution and heterogeneity of coral reefs and covers only a small portion of the taxonomic and phylogenetic breadth of corals. Moreover, there is a yet unexplored, diverse world of cold water corals, possibly hosting novel microbial symbionts underlying unique bioactivities and novel chemical structures (Buhl-Mortensen and Mortensen 2005; Danovaro et al. 2010; Baillon et al. 2014). Indeed, cold water corals face unique challenges, such as strong hydrostatic pressure, absence of sunlight and low temperatures, which act as drivers for unique coral microbiomes (Röthig et al. 2017; Goldsmith et al. 2018; Lin et al. 2023). *Sulfitobacter* strains isolated from cold water corals *Dendrobathypathes* sp. and *Telopathes* sp. show high secondary metabolite biosynthesis potential, harbouring six or more secondary metabolite biosynthetic gene clusters (BGCs) per genome that code, amongst others, for novel terpenes, type I polyketides and non-ribosomal peptides (Lin et al. 2023). Moreover, yet uncultured *Thioglobaceae* symbionts (*Candidatus* Microaerophilica antagonistica) of the temperate, azooxanthellate octocoral *Leptogorgia sarmentosa* were found to harbour unique type III polyketide synthase and bacteriocin (RiPP) BGCs (Keller-Costa et al. 2022).

15.10.2 Leveraging 'Omics' Tools in Coral Microbiome Research to Boost Natural Product Discovery

Although the number of new bioactive compounds described from marine organisms and marine microbial symbionts is continuously increasing (Carroll et al. 2021, 2022, 2023), there is still a long way to go to unveil the full biotechnological potential of these compounds and to translate them into tan-



Leaflet | © OpenStreetMap contributors, CC-BY-SA, Tiles © Esri — Source: Esri, i-cubed, USDA, USGS, AEX, GeoEye, Getmapping, Aerogrid, IGN, IGP, UPR-EGP, and the GIS User Community

Fig. 15.4 World map representing the number of novel compounds (coloured dots) reported from coral-associated microbes in the period from 2018 to 2022 according to sampling location

gible products. Most reports focus on the chemical structure of a novel compound and its synthesis and laboratory production, while limiting the number of bioactivity tests to a minimum. Testing new compounds for all kinds of possible bioactivities without previous insight is a tedious task, wherefore laboratories focus their efforts on those bioassays they have well established and readily available. Thus, many compounds remain without any activity assigned. In this review, around 46% of new natural products (177 compounds) described between 2018 and 2022 were reported as inactive (for the activity test performed) or had no bioactivities described.

Genome inspection is the first solution to tackle this problem (Fig. 15.5). BGC mining has become essential for natural product discovery, allowing the identification of gene clusters responsible for secondary metabolite production and prediction of chemical structures, permitting an early-stage assessment of chemical novelty. Additionally, this type of approach allows to identify possible metabolites that are absent in the chemical extracts of bacterial or fungal isolates, i.e., metabolites whose BGC might be silent under laboratory conditions. Genomics-guided approaches are therefore crucial for a full exploration of versatile secondary metabolism of coral symbionts. The analysis of the genomes of three *Aquimarina megaterium* strains as well as of eight *Roseibium album* strains, isolated from the octocoral *Eunicella labiata*, showed not only the presence of Protein families and Clusters of Orthologous Groups of proteins for symbiosis factors (e.g. ankyrin repeats), but also a variety of BGCs encoding terpenes, polyketides, nonribosomal and ribosomal peptides, among others, revealing a vast potential for natural product discovery (Couceiro et al. 2021, 2022). A recent study by Silva et al. (2022) found robust antimicrobial activities against notorious human pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida glabrata* in several octocoral and marine sponge-associated *Aquimarina* strains and their metabolome profiles suggested the presence of novel cyclic peptides. Moreover, Almeida et al. (2023) identified several, underexplored octocoral-derived bacteria which displayed rich and varied BGC profiles as well as antibacterial and antifungal activity, representing a valuable reservoir for marine drug discovery. Primer-less, shotgun metagenomic sequencing can unveil secondary metabolism features of yet unculturable coral-associated microorganisms as shown in Keller-Costa et al. (2021, 2022). A stronger integration of metagenomics and its

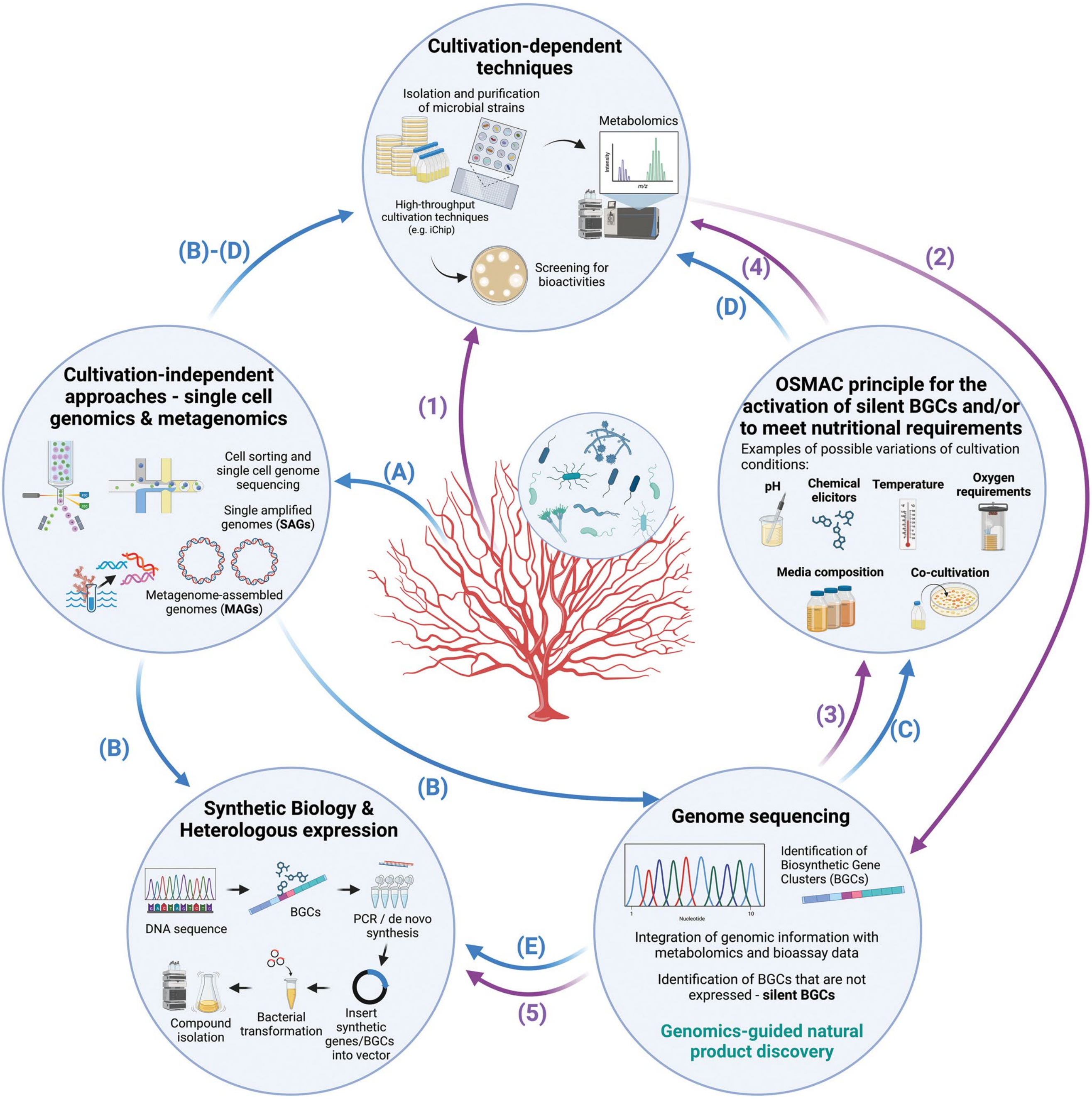


Fig. 15.5 Possible workflows to accelerate discovery of new natural products from coral microbiomes. → **Purple arrows:** Workflows based on cultivation-dependent techniques. Direct cultivation of microbes (*1*) allows for bioactivity screening, metabolomics studies and genome sequencing (*2*). Inspection of genomes along with bioactivity tests to determine the presence of biosynthetic gene clusters (BGCs) and possible application of the OSMAC principle to activate silent BGCs (*3*) and annotation of the novel metabolites obtained with the altered cultivation conditions. Genome sequencing and BGC annotation allows subsequent heterologous expression of certain BGCs in growth optimized strains (*5*). → **Blue arrows:** Workflows based on cultivation-independent approaches. Cell sorting, single cell genomics and/or metagenomics, and the reconstruction of single amplified genomes (SAGs) and/or metagenome-assembled genomes (MAGs) (*A*) give access to the genomics content of unculturable microorganisms (*B*). The MAGs and SAGs can be used to understand the nutritional requirements of uncultured microbes and to achieve their cultivability through custom-designed culture media (*C*), thus obtaining novel isolates for bioactivity screening and metabolomics studies (*D*), and/or to obtain the BGCs sequences of unculturable microorganisms and harness the underlying secondary metabolites through synthetic biology and heterologous expression (*E*) (figure created in BioRender)

coupling to synthetic biology and heterologous expression can provide access to the secondary metabolism of the uncultured fraction of coral symbionts in the future (Fig. 15.5).

15.10.3 Unlocking the True Biosynthetic Potential of Coral-Associated Microorganisms

In microorganisms, secondary metabolism frequently occurs in stationary growth phases, with a shift in the efforts of the organisms from reproduction to the production of auxiliary metabolites (Chevrette et al. 2020). The molecules produced in these stages serve several different ecological purposes, generally conferring advantages to the producing organism (Katz and Baltz 2016), and, indeed, often have promising unrelated activities with potential uses for society (Yuan et al. 2016). However, the production of these metabolites is generally very energy and resource consuming with many of the genes involved in these processes being silent until a specific environmental trigger activates a specific pathway. To harness the full potential of microbial secondary metabolites production it is imperative to understand these processes and employ efforts in high throughput screening of cultivation conditions to activate silent BGCs. In 2002, a team of researchers postulated the “One Strain Many Compounds” (OSMAC) principle (Fig. 15.5), establishing the systematic exploration of cultivation parameters, such as temperature, aeration, media composition or addition of chemical elicitors with the purpose of increasing the number of secondary metabolites produced by one microbial strain (Bode et al. 2002). The same principle was later adapted and applied to marine microorganisms (Romano et al. 2018; Pan et al. 2019). For example, testing 22 growth media with different carbon and nitrogen sources and concentrations combined with genomics and untargeted metabolomics led to the identification of new glycolipids with significant antiviral activity, produced by *Rhodococcus* sp. strain isolated from marine sediment (Palma Esposito et al. 2021). The OSMAC principle also successfully uncovered new metabolites from coral-derived microorganisms. The anticancer compounds cochliobopyrones A and B from hexacoral-derived *Cochliobolus lunatus*, previously mentioned in this chapter, resulted from distinct metabolite profiles obtained after co-incubation with DNA methyltransferase inhibitors (Wu et al. 2019). *Pseudoalteromonas* sp. strain OT59 isolated from the gorgonian *Leptogorgia alba* produces the polyketide ateramide A, a compound that displays light-dependent antifungal activity against coral pathogens (Moree et al. 2014). Strain OT59 produces larger quantities of alteramide A in the dark, while light exposure inactivates the compound through photo-induced cyclization, illustrating how even the simplest changes in cultivation conditions can have great impacts on secondary metabolite production and activity.

15.11 Final Remarks

Coral-associated microorganisms hold great promise for the discovery of novel chemical compounds with potential applications in the most diverse industries, such as the health, agriculture or maritime sectors, and their metabolites emerge as sustainable alternatives to previously used, toxic or obsolete molecules. However, the field of coral microbiome biotechnology has yet many obstacles to overcome. We need to expand the breadth of bioactivity tests applied to each new molecule which can only be achieved through genuine collaboration between multidisciplinary laboratories with complementary expertise and screening systems. Restoration and conservation efforts are also important to retain these organisms, not only for their ecological importance but also due the biotechnological products, and other economic assets, they provide. Ensuring universal access to rich and accurate compound-associated metadata to obtain reliable information about e.g., sampling location and coral host species is key to counteract current sampling-associated biases in coral microbiome oriented natural products research. Advanced MultiOmics approaches that facilitate high throughput screenings of promising microbial strains are further necessary to leverage the full coding potential of coral-associated bacteria and fungi and to increase compound numbers and structural novelty. Given that drug approval demands extremely high, long-term investments with 90% of the natural products notwithstanding the scrutiny of the drug discovery and approval pipeline (Sun et al. 2022, p. 90), the scientific community should consider strengthening future research on non-medical industrial applications, such as the development of new ecofriendly antifouling substances for the maritime sector, biocatalysts in waste treatment and recycling/upcycling processes, or nature-based cosmetic ingredients and nutraceuticals. This can allow us to fully harness the benefits of this untapped reservoir of new molecules, accelerate the marketability of coral microbiome derived compounds, and promote sustainability in harmony with the United Nations sustainable development goals.

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Building Coral Reef Resilience Through Assisted Restoration

16

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Abstract

Based on concepts and data discussed throughout this book, here we introduce the term "Assisted Restoration" (AR) as the sum of approaches required to deliver effective ecological restoration. AR signifies a multidisciplinary strategy with which innovative tools are integrated into routine practices of coral restoration to build sustained resilience for increased survival in a changing world. Coral microbiomes are arguably central targets for active intervention strategies due to their malleability, as well as for monitoring approaches because of their biomarker capacity, serving as indicators of healthy ecosystems. We highlight the role of customized and integrated approaches to optimize coral reef interventions and indicate how artificial intelligence (AI) and standardized data collection methods have the potential to revolutionize the monitoring and analysis of coral reef health under an Assisted Restoration approach. Such an integrative approach is critical to advance ecosystem-scale restoration and is directly connected to planetary and human health under the One Health concept.

Keywords

Coral reef · Holobiont · Metaorganism · Microbiome manipulation · Probiotic therapy · Microbiome stewardship · Reef restoration · Artificial intelligence

16.1 The Prospect of Microbial-Driven Reef Ecosystem Restoration

Microorganisms connect most entities in our biosphere and drive biogeochemical cycles that shape our planet. Microorganisms therefore represent suitable and primary targets for ecosystem health assessment and active intervention, notably given that microbiome structure and function shape the structure and function of ecosystems and their contained animal, plant, and fungal biodiversity (Peixoto et al. 2022). Targeted management of the microbiome as a means to safeguard ecosystem and organismal health is termed "microbiome stewardship" (Peixoto et al. 2022). The concept of microbiome stewardship also recognizes the importance of microbial communities in sustaining human health and emphasizes the need to protect microbiomes, and thereby the ecosystem, through policy and active intervention. Much of what we know about coral reef microbiomes and the mechanisms of interactions within the coral holobiont is likely relevant for other organisms and ecosystems and *vice versa* (Peixoto et al. 2021). Given that coral reefs are among the first marine ecosystems on the brink of ecological collapse, there is an urgent need to rapidly advance frameworks for effective ecosystem restoration for coral reefs (Peixoto et al. 2024a). At present, reef restoration predominantly takes a macro-ecological lens from the viewpoint of coral propagation and replanting, leading to major gaps in factors and processes that may ultimately contribute to ecological

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recovery, including micro-ecological processes and the underlying microbial communities.

Quite different from the traditional view and approach of administering antimicrobials for the treatment of disruptions caused by microbes (Yahav et al. 2021), the concept of microbiome stewardship focuses on a more holistic approach with a focus on prevention, taking into consideration the maintenance of healthy microbiomes (beneficial members) as a gateway to organismal well-being and stress resilience, and thereby, ecosystem robustness, which is also becoming a new directive in human medicine (Kirchhelle and Roberts 2022). Despite the compelling notion of 'fixing the microbiome to fix the ecosystem', understanding and restoring microbiome function is a daunting task. This is due to the inherent complexity and dynamic assemblage of microbiomes and our lack of understanding of the ecological mechanisms that govern microbiome assembly, change, and evolution, let alone uncertainties regarding the structure of pristine or undisturbed microbiomes (Peixoto and Voolstra 2023). Details regarding the challenges and opportunities are discussed elsewhere in depth (Voolstra et al. 2021; Maire and van Oppen, 2022; Voolstra et al. 2024; Mohamed et al. 2023; Peixoto et al. 2022), as well as in Chapter 13. Here we touch upon the prospect of coral reef restoration (which spans the many modalities of restoration actions) (e.g. Hein et al. 2021) and how rapidly advancing data approaches, such as artificial intelligence (AI), can contribute to the notion of assisted restoration (AR) and support evidence-based restoration to foster efficacy and long-term success (Peixoto et al. 2024b).

16.2 The Dawn of the Age of Data Science

New technologies are accelerating the capacity for data collection in coral reef systems. Data science will play a pivotal role in informing—and, in turn, transforming the effectiveness and scale of—reef conservation and restoration (Fig. 16.1) (Voolstra et al. 2025; Goergen 2020; Voolstra et al. 2021). Automation is extending the temporal and spatial scales with which reef ecological and process-based data can be collected, overcoming the challenges of in-water dive time, and in turn, rapidly moving operational bottlenecks towards how large datasets can be stored and re-accessed, analyzed, and modeled. Such challenges further extend to environmental and omics data (Voolstra et al. 2025). Whilst in their infancy for reefs, Machine learning (ML) and artificial intelligence (AI) approaches hold great promise to advance reef health predictions, threat assessments, and optimization and decisions-making underpinning restoration strategies based on complex datasets (Staab et al. 2024). In many ways, data is rapidly becoming the new "economy" critical to inform evidence-based and effective reef management and conservation. This is the case for microbiome research—and how it can aid reef management—and may even develop faster for other branches of reef science (Peixoto et al. 2024b). We first highlight the different (semi-)automated approaches that are emerging for data collection and reef monitoring and then how they can be used in the real world to inform and improve the efficacy of restoration approaches.

Automated and, where possible, standardized data collection in coral reef monitoring will need to incorporate a diverse array of variables, including temperature and chemical sensor data, acoustics and visual data stemming from satellites, drones, divers, or automated underwater vehicles (Voolstra et al. 2025). Integration will facilitate comparison and efficient mapping and monitoring of coral reefs, significantly advancing our knowledge through automated reef surveys (Voolstra et al. 2025; Voolstra et al. 2021; Goergen 2020). Achieving this goal requires the development of advanced data storage architectures, robust data management systems, efficient data transmission protocols, and improved data sharing and accessibility frameworks. These technological advancements are essential, as the current bottlenecks in data handling and analysis often limit the progress we can make in coral reef research and conservation.

Remotely sensed imagery plays a pivotal role in efficiently mapping and monitoring benthic habitats. Advanced machine-learning algorithms have been implemented to classify satellite-retrieved images to identify coral reefs and other benthic habitats (Burns et al. 2022). Such algorithms are also capable of predicting fish species richness (Knudby et al. 2010). Preprocessing in the form of water column correction and sunglint removal are important steps to enhance image quality and accelerate machine learning (Nguyen et al. 2021). Airborne imagery, usually obtained via drones, offers accessible means for local reef surveys and research with a means to map benthic habitats (Nababan et al. 2021), predict coral thermal tolerance (Drury et al. 2022), and track migratory organisms (Sankaran 2024). Similarly, fixed underwater observatories (FUOs) (Osterloff et al. 2016) enable continuous automated monitoring of reef environments through various sensors and tools (chemical sensors, image/video, etc.) and time-series data modeling, offering insights into changes over time. Increasing deployment of automated underwater vehicles (AUVs) represents a major leap forward providing a more efficient means than divers to assess and monitor reefs (Davis and Paneerselvam 2023) and, when integrated into machine learning (ML) frameworks, can make more informed decisions in where to collect data (including obstacle avoidance) (Giguere et al. 2009). Close-range underwater images captured by AUVs can be analyzed by classification and image annotation (CNN) algorithms for tasks such as biomes classification (Lumini et al. 2023), coral species identification (Mahmood et al. 2019; Sharan et al. 2021), and coral health assessment (Macadam et al. 2021; Narayan and Pellicano 2021). For coral species classification, a detailed analysis of whether the image shows the texture or the structure of the coral has to be

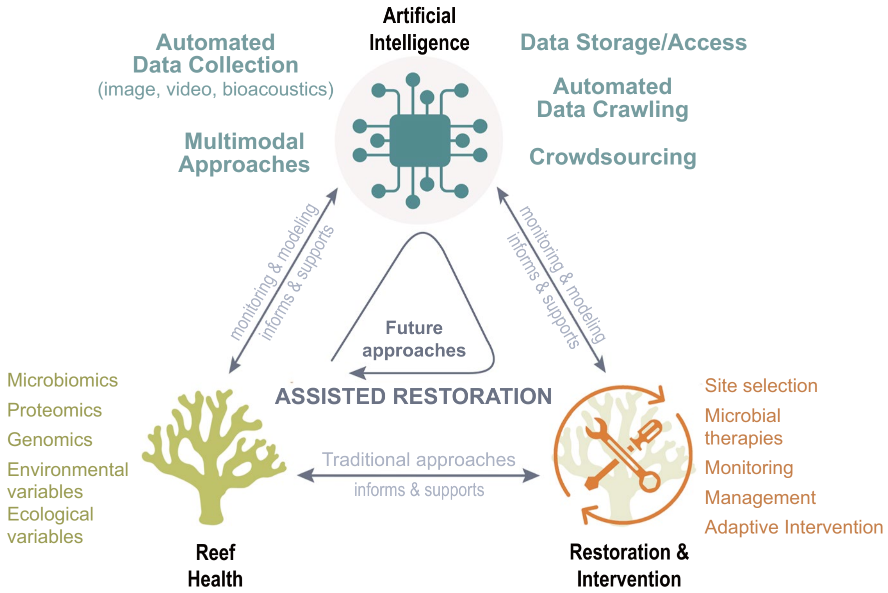

Fig. 16.1 Artificial intelligence (AI) improves coral research and restoration and intervention approaches in various ways. It allows the monitoring, analysis, and modeling of reef health with a variety of data collection and modeling tools. Reef health research informs restoration and intervention approaches. AI supports monitoring restoration and intervention success, thus enabling evidence-based decision-making and approach refinement to maximize recovery trajectories. Connected health monitoring and future prediction networks utilize AI analysis to suggest active intervention (restoration) in cases where health/coral cover is at risk (Voolstra et al. 2025; Voolstra et al. 2021)

performed first (Gómez-Ríos et al. 2019a, b), but various online platforms are in place that employ ML approaches to assist benthic reef survey data (Williams et al. 2019; Colin et al. 2024). Such approaches will likely be further enhanced by the use of multispectral and hyperspectral imaging technologies (Young et al. 2018; Huot et al. 2023). Whilst these various capacities offer great transformative potential, they remain in an extremely early technological readiness level (TRL). For example, AI/ML effectiveness will rest on the training datasets available for species recognition and health assessment, which are in their infancy—in part reflecting a lack of robust and/or consistent species taxonomic or functional libraries.

Photos and video capture has now become routine for reef science enabling “structure from motion” photogrammetry to better visualize reef form and function; this process involves machine learning and creates detailed 3D structures of coral reefs from visual data (Zhong et al. 2023; Sauder et al. 2023), allowing researchers to quantify coral loss (Kopecky et al. 2023) and predict fish distribution (Pittman and Brown 2011). Again, such opportunities are in a very early phase of robust and routine deployment where data storage, access, and processing govern the scales and resolution with which 3D imagery can be re-created and analyzed. For example, capturing coral growth requires that images are spatially robust over time, and with the image and point-cloud resolution needed to visualize change.

Beyond visual and spectral data, automatically detected bioacoustics provides an additional source of information, helping to detect subtle changes in reef ambient noise (Ozanich et al. 2019) and to distinguish between sounds made by different marine species (Ozanich et al. 2021). When combined with other types of data in a multimodal approach (Kline et al. 2021) such diverse datasets can yield comprehensive models that enhance our understanding of reef dynamics. However, developing tools built around data mining platforms (e.g., large language models) will be critical to leverage data as it continues to become ever more expansive and complex. Automated data crawling/scraping can help gather publicly available data from the internet, accelerating research, and broadening data accessibility, although it should be noted that how well this returns meaningful data rests on how well publicly available data has been robustly annotated. Crowdsourcing platforms, guided by AI algorithms, further expand data collection by engaging citizen science, therefore aggregating extensive datasets efficiently (Voolstra et al. 2025; Narayan and Pellicano 2021). A recent example is Australia’s “Great Reef Census” a large-scale citizen science initiative aimed at collecting and analyzing images of the Great Barrier Reef to assess its health and biodiversity. The project was launched in 2020 and is led by the conservation organization Citizens of the Great Barrier Reef, in collaboration with scientific institutions, reef operators, and volunteers.

Technological improvements can clearly collectively generate a robust framework to inform conservation and restoration. By integrating diverse data sources and innovative analysis techniques, we can accelerate our predictive capabilities regarding coral reef decline and enhance strategies for coral reef restoration and rehabilitation, ultimately sustaining coral reef health and ecosystem stability. Equally important, these data can be used to build models for coral health and resilience. Prediction analysis can be used to predict future bleaching events and analyze features that are connected to bleaching (Boonnam et al. 2022). In addition to the resilience of coral reefs, connectivity and biodiversity can be analyzed through network analysis (Novi and Bracco 2022). With the help of ML, coral extinction risk can be modeled by incorporating coral fossil data (Raja et al. 2021). A combination of anthropogenic and biophysical predictors of benthic communities (including coral cover, fish biomass, and turf cover) allows a quantitative comparison of their impact (Jouffray et al. 2019; Umanandini et al. 2021). Quite simply, we are at the stage where data volume and analytical capacity govern how we can improve our understanding of reef systems needed to inform their status and hence robust decisions to secure their future, but we cannot save all reefs and some difficult decisions need to be made. Data science approaches can provide objective, evidence-based suggestions to guard and justify decisions being made.

Restoration is one integral part of ensuring a future for coral reefs (Peixoto et al. 2024a; Suggett et al. 2024). Cost, motivation, need, and impact of restoration projects are based on visual assessments, monitoring, and/or predictions that need to be available in a feasible time frame for interventions to be effective (Bayraktarov et al. 2019; Goergen 2020; Lange et al. 2024). Although, in many cases, the damage and coral reef declines are already visible and undeniable, AI/ML can help to continue to forecast further impacts and support the selection of restoration sites or reserves (Leslie et al. 2003). ML can, for example, use the available data to generate models that can predict suitable locations predetermined for conservation (McClanahan 2023) and restoration. On a parallel note, these tools can also help quantify the importance of coral reefs for fishing industries (McClanahan et al. 2023), tourism, coastal protection, and other economic activities that can grant more support from stakeholders and reinforce a quest for more legislative reef protection.

16.3 Assisted Restoration: Customizing Resilience- and Ecosystem-Based Coral Reef Restoration

One Health is a holistic approach recognizing the interconnectedness of human, animal, and environmental health (One Health High-Level Expert Panel (OHHLEP) et al. 2022). Applied to coral reefs, it considers the interplay between the coral and the reef microbiome, the health of corals, marine life, and, ultimately, human communities (Sweet et al. 2021; Peixoto et al. 2022). Addressing coral health in the broader context of ecosystems and human well-being and integrating tools to enhance coral resilience are essential for the establishment and retention of newly restored or rehabilitated reefs.

As extensively discussed in this book, coral reef-associated microbiomes are prime stress indicators and targets for customized therapies to increase resilience and recovery. Whilst there is a clear promise for AI/ML for coral reef science as outlined above, this also fundamentally applies to microbiome research—and its role in guiding more effective reef management—on multiple levels. ML allows effective preprocessing, analysis, and predictive modeling of microbiome datasets that are typically elusive. For example, the microbial communities of seawater surrounding coral reefs have been found to be predictive of temperature and eutrophication state (plant and algal growth), while host-associated microbiomes seem to be much more stable (Glasl et al. 2019). Machine learning-based tools can be used to identify and track specific taxa, functions, patterns, and correlations. VirFinder, for example, is used to identify viral sequences in marine metagenomes (Ponsero and Hurwitz 2019), while Coracle (Staab et al. 2024) is an innovative ML-based feature selection framework built to identify key features in sparse and wide datasets. Coracle can, for example, support the search for coral probiotics or identify genes connected to coral bleaching by first modeling and predicting a target variable (e.g., ED50 standardized thermal tolerance thresholds) in a supervised ensemble approach and then analyzing the importance and robustness of its features (e.g. microbiomes ASVs, genes) (Staab et al. 2024; Voolstra et al. 2020; Evensen et al. 2023).

Integration of microbial-driven coral health enhancements and AI/data science - amongst other factors (e.g., engineering solutions for automation) - into traditional restoration practices to improve local conditions under the One Health approach provides the foundation for a more comprehensive framework for safeguarding coral reefs, here termed "Assisted Restoration" (AR) (Fig. 16.2). This multidisciplinary concept is critical to addressing the complex and interrelated challenges facing these unique and fragile ecosystems in an efficacious manner. A practical example would be the customized combination of different strategies to create a resilience-based restoration framework (i.e., where the intent is to boost the resilience of restored taxa, ecological communities, ecosystems) (Voolstra et al. 2021). For instance, structures that can be used to either grow coral stock (e.g., nurseries) or restoration structural frameworks themselves (e.g., the MAARS Assisted Reef Restoration System) (Lamont et al. 2022; Razak et al. 2024) can be used to restore thermally superior coral colonies following prior screening (Voolstra et al. 2021), integrated with AI-informed

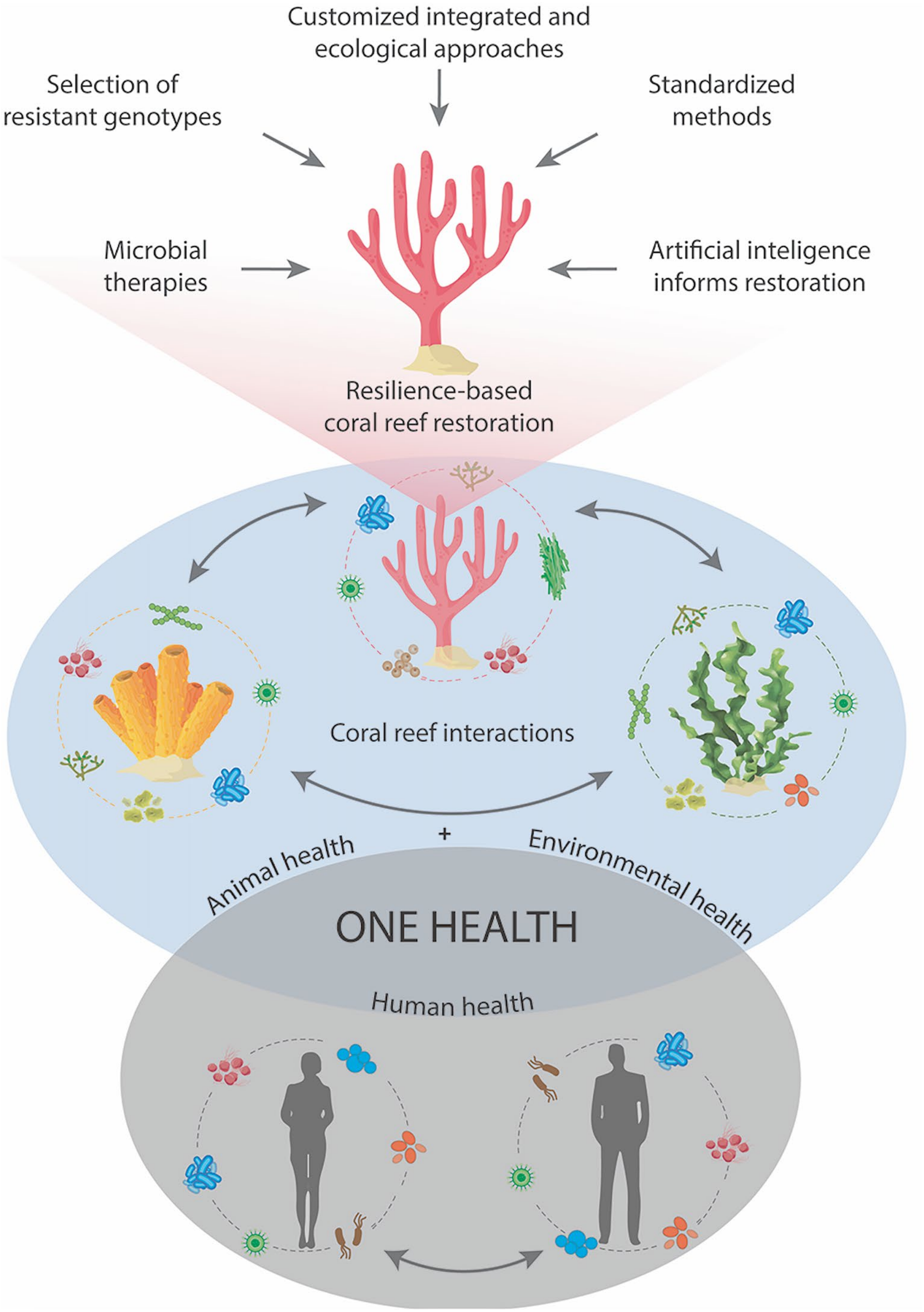

Fig. 16.2 The concept of Assisted Restoration (AR) represents the customized and integrated use of targeted active interventions, such as microbiome stewardship and AI/data science approaches, to build sustained resilience in conservation/restoration efforts ultimately producing cascading benefits for reef ecosystems and beyond, contributing to the broader concept of One Health

microbial therapies (Staab et al. 2024) or other interventions (Van Oppen et al. 2015) to increase long-term coral survivorship (Santoro et al. 2021) and growth (Zhang et al. 2021; Moradi et al. 2023).

Coral mortality caused by fragmentation during restoration efforts can be potentially reduced through microbiome stewardship. The fragmentation process itself causes tissue damage (Bowden-Kerby 2001), which can become an entry point for microorganisms or cause dysbiosis-driven disease outbreaks (Brandt et al. 2013). The use of probiotic cocktails composed of microorganisms with antagonistic traits against major pathogens could increase survival and acclimatization rates of coral to new substrates in land-based nurseries. Additionally, probiotics can be applied during the first weeks after coral outplanting in restoration efforts to increase survivorship and/or during heatwaves to ensure the retention of restored corals, providing ready-now examples of AR.

As alluded above, the selection of coral colonies with superior thermal tolerance as source material for restoration can be prioritized (Voolstra et al. 2021) and further enhanced (van Oppen et al. 2015) to increase survival under future climate scenarios. Coral thermal tolerance can be quickly and efficiently compared through the use of standardized methods (Voolstra et al. 2025), such as the pioneering Coral Bleaching Automated Stress System (CBASS) (Evensen et al. 2023; Voolstra et al. 2020), in restoration and population ecological approaches (Cunning et al. 2021; Naugle et al. 2024; Klepac et al. 2024). Colonies classified as inferior thermally tolerant than the ones selected for the restoration effort can also be included to maintain a diverse genetic pool. Such approaches can be combined with the restoration and management of other coral reef organisms.

A practical example of such a combination approach would be the integration of sponges (phylum Porifera) and their associated microbial communities into coral restoration efforts. Sponges are benthic, sessile, and efficient filter-feeding animals (Weisz et al. 2008) that harbor microbial communities in varying abundances, including bacteria, archaea, viruses, microeukaryotes, and fungi (Taylor et al. 2007; Thomas et al. 2016). In oligotrophic environments such as coral reefs, the efficient filter-feeding capacity of sponges is crucial for maintaining high biodiversity by recycling and enhancing the available organic matter to benthic detritivores, thus supporting the trophic chain (de Goeij et al. 2013). Further, incorporating sponges into reef restoration efforts can be beneficial by providing additional food for corals (Slattery et al. 2013; Reigel et al. 2024), improving water quality, and controlling pathogen abundance through means of their filtration capacities (Gökalp et al. 2021; Aguilo-Arce et al. 2023). Sponges can accumulate (or degrade) anthropogenic pollutants (such as pathogenic microorganisms or heavy metals), the latter being strongly linked to their microbial symbionts (Longo et al. 2016; Marzuki et al. 2023), which make these animals key players to be included in restoration projects.

In the context of coral restoration, a ‘mixed mode’ cultivation strategy could be conceived by propagating corals and sponges in nurseries and coral restoration frameworks. Sponges from the surrounding reef areas could serve as donors for sponge explants, which would then be attached to coral restoration structures. This methodology is cost-effective as it intends to use the existing restoration infrastructure for corals, and the healing process of the sponges before attachment could be conducted using simple aquaculture methods (Bierwirth et al. 2022; Amato et al. 2024).

To achieve long-term conservation and restoration success, science-guided Assisted Restoration (AR) must be integrated with long-term efforts to improve local conditions to further enhance and sustain resilience. Interventions to enhance the stress tolerance of corals are unlikely to succeed without addressing local environmental conditions (Knowlton et al. 2021; Donovan et al. 2021). Among local factors, fish biomass and water quality are shown to greatly affect reef resilience (D’Angelo and Wiedenmann 2014; Haas et al. 2016; Robinson et al. 2020; Vega Thurber et al. 2014). Presently, this is rarely achieved (Boström-Einarsson et al. 2020).

16.4 Microbiome Restoration and Rehabilitation as an Integral Part of Assisted Restoration (AR) and One Health

An AR approach involves leveraging the diverse tools at our disposal to advance ecological restoration. Microbes play crucial roles in nutrient cycling, disease resistance, and overall reef health and, accordingly, can have a key role in augmented active interventions to build resilience into restoration. In Chapter 13, we present coral host-based microbial therapies that can rehabilitate the coral holobiont. Here, we apply such notions to the ecosystem scale under an assisted restoration (AR) framework. Potential strategies include inoculating reefs with beneficial microbes, promoting coral-algal symbioses, and enhancing nutrient recycling. By harnessing microbial dynamics, coral restoration in combination with microbiome stewardship aims to improve the resilience and vitality of entire reef ecosystems. It should be acknowledged that counter to the hesitation by many to ‘manipulate’ natural environments, most ecosystems and thereby microbiomes are already altered and are continuously being manipulated by human impacts, usually towards a more pathogenic assemblage (Peixoto and Voolstra 2023), signified by highly uneven and less diverse microbial communities (Peixoto et al. 2022; Berg et al. 2020). Decades may be needed for degraded ecosystems to fully reestablish critical functional traits, the restoration of which can be accelerated and improved through microbiome stewardship (Gellie et al. 2017; Andras et al. 2020). Given the current trajectory, one may argue it is too late for nature to heal itself, with significant losses being expected in the coming years (Knowlton et al. 2021). Active direct or indirect interventions may therefore be a necessary complement to any restoration effort to become long-term sustainable. Here, it is important to make the distinction between what is possible now and what will be possible in the future, considering the impact that data science and AI will have on our ability to conserve and restore more effectively and at ever-increasing scales. We need to make better, more informed decisions faster to ensure that a sufficient number of foundational species survive to assist in the long-term recovery of ecosystems (and microbiomes), once (and when) climate neutrality is reached (Fig. 16.2).

16.5 Conclusions

In this book, we explore coral microbiomes, their diversity, function, and interactions with corals and the reef ecosystem, indicating their key role in coral health and resilience. Here, we close the "Coral Reef Microbiome" book by incorporating such crucial role of coral- and coral reef-associated microbiomes into a bigger and more complex picture. We do this by describing a new era of coral reef resilience-based restoration, where the study of coral microbiomes and the use of microbial therapies play a fundamental role—combined with the use of a multitude of other innovative tools. We propose the term Assisted Restoration (AR) to define a multidisciplinary and integrative approach involving augmented active interventions aimed at building and enhancing resilience into coral restoration. By harnessing innovative tools such as microbiome manipulation, AI-driven data analysis, and customized restoration strategies, AR offers a comprehensive and science-based framework for addressing the complex challenges that coral reefs face today. The importance of coral-associated microbiomes as ecosystem health indicators and targets for restoration efforts cannot be overstated. Microbiome stewardship, among other concepts/tools, integrated with advanced data science approaches provides a powerful means to optimize restoration outcomes and build ecosystem resilience. While coral reef restoration remains a relatively young field, it is rapidly maturing through the incorporation of cutting-edge technologies and methodologies, constantly expanding the horizon of what may be achievable in terms of scale, effort, and long-term sustenance. The One Health approach, which emphasizes the key relationships between humans, other organisms, and environmental health, underscores the broader implications of coral reef restoration for other ecosystems and human well-being globally. Moving forward, the successful implementation of AR will require ongoing collaboration across disciplines, continuous innovation, and the development of flexible and standardized frameworks for data collection and analysis. As we enter an era where data science becomes increasingly central to conservation efforts, the ability to make informed, evidence-based decisions will be paramount. Ultimately, AR represents a crucial step toward safeguarding the future of coral reefs and the countless species and communities that depend on them.

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