Understanding the Coral Holobiont through Science and Scuba

Steve V. Vollmer, Andrew C. Baker, Mary-Alice Coffroth, C. Drew Harvell, and Mónica Medina

ABSTRACT. Reef-building corals are a holobiont composed of the coral animal host and its associated eukaryotic and bacterial microbes. Symbiotic dinoflagellates in the genus *Symbiodinium*, which form the basis of the coral–algal symbiosis and provide the coral host with most of its nutrition, are one of the most familiar members of the coral holobiont. Yet reef-building corals also possess diverse communities of bacteria that play important roles in processes such as nutrient cycling and coral immunity. Understanding the complex relationships between the coral, its algal symbionts, and associated microbes is critical because breakdowns in these relationships result in coral bleaching and coral disease outbreaks, both of which are increasing due to global climate change. Here we review recent advances in scuba-based research on the coral holobiont that have expanded our understanding of coral–algal and coral–microbe relationships as well as the role of the coral host in these interactions.

INTRODUCTION

Tropical coral reef ecosystems harbor well known and arguably unrivaled biological diversity that supports the marine world (Knowlton, 2001a, 2001b; Hughes et al., 2003). The macroscopic, or visible, diversity of life on coral reefs, including the array of tropical fish, seaweeds, and invertebrates, is readily apparent to anyone who has snorkeled or scuba dived on reefs. This visible biodiversity tends to capture the most scientific and public interest. Yet there is a greater diversity of microorganisms on reefs that goes unseen (Rohwer et al., 2002; Rosenberg et al., 2007; Dinsdale et al., 2008a, 2008b; Bourne et al., 2009; Ainsworth et al., 2010). This hidden diversity of microbes is arguably more important to the function of coral reef ecosystems because these microorganisms are instrumental in critical ecosystem services like nutrient cycling (Rohwer et al., 2002; Alongi and McKinnon, 2005; Wegley et al., 2007; Thurber et al., 2009).

Reef-building corals are the foundation of coral reef ecosystems. These reef-building coral species are host to an amazing diversity of organisms including representatives from all three domains of life (eukarya, bacteria, archaea) and viruses (Rohwer et al., 2002; Rosenberg et al., 2007; Ainsworth et al., 2010). Eukaryotic organisms associated with the coral host include the well-known, phototrophic, symbiotic dinoflagellates in the genus *Symbiodinium* (Rowan, 1991; Trench, 1993; Baker, 2003; Stat et al., 2006), which translocate fixed carbon to the host (Muscatine, 1973; Muscatine et al.,1981; Falkowski et al., 1984; Edmunds and Davies, 1986) as well as other eukaryotes associated with the coral tissue and skeleton, including endolithic algae, fungi, and sponges (Rohwer et al., 2002; Rosenberg et al., 2007; Bourne et al., 2009). Corals house a high abundance and diversity of bacterial and archaeal groups in their tissue, surface mucus, and skeleton (Rohwer et al., 2002; Wegley et al., 2004; Rosenberg et al., 2007; Bourne et al., 2009).

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Manuscript received 25 January 2012; accepted 5 March 2013.

These bacteria and archaea can benefit the coral by cycling nutrients (Rohwer et al., 2002; Lesser et al., 2004; Wegley et al., 2004; Beman et al., 2007; Wegley et al., 2007) and producing antimicrobial compounds (Kelman et al., 1998; Ritchie, 2006; Nissimov et al., 2009). However, some of the coral-associated bacteria are also detrimental to the coral, including pathogenic bacteria that cause disease (Kushmaro et al., 1996; Rohwer et al., 2002; Sutherland et al., 2004; Harvell et al., 2007; Rosenberg et al., 2007; Bourne et al., 2009; Ainsworth et al., 2010).

The collection of organisms associated with and including the host coral animal has been called the coral holobiont (Rohwer et al., 2002). The concept of the coral holobiont (Rohwer et al., 2002) originated in part from the need to understand the newly discovered diversity of microorganisms on corals in a more comprehensive or holistic way, (i.e., by viewing the coral as the sum of its parts—the animal host plus all of the associated eukarya, bacteria, and archaea). This expanded view of the coral organism began to emerge as it became apparent that the symbiotic dinoflagellates within corals, which were once thought to be one species, *Symbiodinium microadriaticum* (Taylor, 1971, 1974), actually comprised many diverse lineages (or clades) of *Symbiodinium* (Rowan, 1991; Rowan and Powers, 1991), even within a single coral (Rowan and Knowlton, 1995; Rowan et al., 1997). The diversity of algal symbionts led to the view that the coral and its algal symbionts represent a holosymbiont (Iglesias-Prieto and Trench, 1997). Rohwer et al. (2002) further defined the coral holobiont to include the coral host, its symbiotic algae, and other associated microbes.

The coral holobiont concept not only represents an assemblage of microbes and a host, but also allows us to better understand how the associations among the groups composing the coral holobiont benefit each other, as well as how breakdowns in these associations might impact the coral and the holobiont community (Figure 1). The holobiont perspective helps to solidify a departure from the earlier tendency in the study of coral biology to focus separately on each component—coral, symbiont, and other microbes. The two most familiar symptoms of breakdowns in the associations between the coral host and its associated microorganisms include (1) coral bleaching caused primarily by the breakdown in the coral–algal symbiosis (Brown, 1997; Hoegh-Guldberg, 1999; Douglas, 2003), and (2) coral disease resulting from the breakdowns between the coral and its associated bacteria (Sutherland et al., 2004; Harvell et al., 2007; Rosenberg et al., 2007; Bourne et al., 2009).

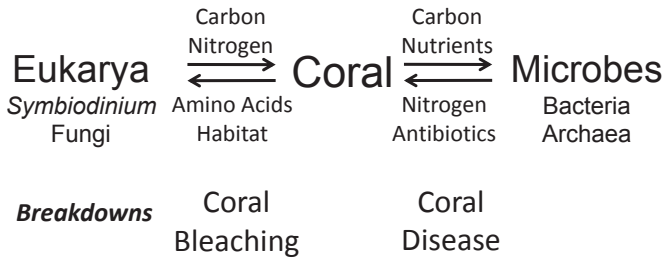

FIGURE 1. Schematic view of the coral holobiont (modified from Rohwer et al., 2002, and Thurber et al., 2009) showing some of the potential benefits and breakdowns between the coral host and its associated eukaryotes, bacteria, and archaea.

The goal of this paper is not to provide a comprehensive review of the literature on the coral holobiont since there are already many excellent and recent reviews on the coral–algal symbiosis (e.g., Baker, 2003; Stat et al., 2006) and coral–microbe interactions (e.g., Sutherland et al., 2004; Rosenberg et al., 2007; Bourne et al., 2009; Ainsworth et al., 2010). Instead, our goal is to highlight recent findings, as well as to characterize the state of knowledge of the coral holobiont, including knowledge gaps that warrant further investigation. Scuba diving research has played a prominent role in all of this work, particularly because it allowed for the experimental manipulation and collection of specimens from the reef environment. We begin by discussing recent advances in our knowledge of the nature of the coral–algal symbiosis, and in particular their ontogeny. We then discuss recent advances in understanding coral–microbe interactions. We conclude by highlighting the role of the coral host as a key member of the coral holobiont.

THE CORAL–ALGAL SYMBIOSIS

All reef-building corals exhibit mutualistic associations with symbiotic dinoflagellates in the genus *Symbiodinium*. These algae were originally identified as *Gymnodinium*-like dinoflagellates by Kawaguti (1944), axenically cultured by McLaughlin and Zahl (1959), and formally described (from the scyphozoan *Cassiopeia* sp.) as *Symbiodinium microadriaticum* by Freudenthal (1962). Despite early cautions to the contrary (e.g., McLaughlin and Zahl, 1966), all symbiotic dinoflagellates were initially classified as members of a single pandemic species adapted to life in a symbiotic state (Taylor, 1971; Taylor, 1974). However, beginning in the mid-1970s evidence drawn independently from a variety of approaches (biochemical, physiological, behavioral, morphological, and genetic) indicated that these dinoflagellates were in fact unusually diverse. Then, in the 1990s, our understanding of diversity in *Symbiodinium* was revolutionized by the application of contemporary PCR-based molecular genetics. The use of scuba allowed scientists to collect a wide variety of symbiotic hosts from a broad range of habitats and environmental conditions. These two factors greatly improved our understanding of how *Symbiodinium* diversity can influence the physiology and ecology of various hosts, particularly corals.

Algal Diversity and Distribution

Rowan and Powers (1991) were the first to PCR-amplify and sequence *Symbiodinium* from reef corals. They recognized

three distinct clades of *Symbiodinium* (named A, B, and C) and demonstrated that the genetic distance between these clades was comparable to that between some nonsymbiotic dinoflagellate orders. In the 20 years since these articles appeared, further evidence of the extraordinary diversity of this genus has accumulated, with six additional clades now recognized: D (Carlos et al., 1999), E and F (LaJeunesse, 2001; Pochon et al., 2001), G (Pochon et al., 2001), H (Pochon et al., 2004), and I (Pochon and Gates, 2010). However, of the nine clades of *Symbiodinium* (A–I) that have been documented to date, only six have been identified from corals: A, B, and C (first recorded by Rowan, 1991), D (Baker, 1999; see also Rowan, 1991; Baker, 2003), F (LaJeunesse, 2001), and G (Van Oppen et al., 2005). The majority of these studies depended on the use of scuba to collect specimens (Figure 2), and the ready access to diverse specimens obtained using this technology has played an important role in these discoveries. Previously, *Symbiodinium* researchers tended to be restricted to working on cultures, which are highly selected and thus can limit studies of diversity by favoring symbionts that can be cultured over others (Santos et al., 2001).

Our knowledge of how different *Symbiodinium* are distributed within and among different coral species has progressed rapidly over the last decade. Additional clades have been documented, and the diversity of subcladal *Symbiodinium* types within some

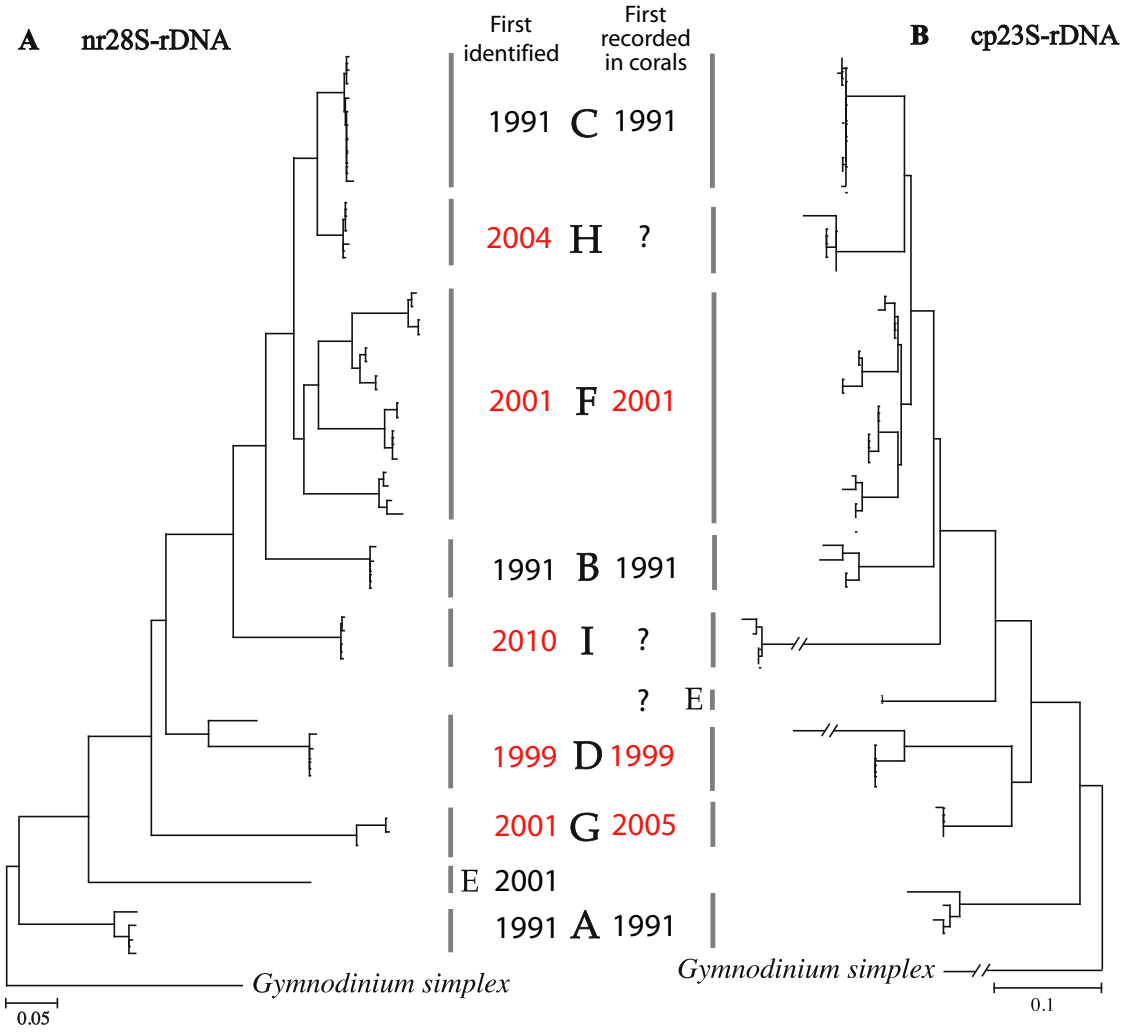

FIGURE 2. Phylogenetic reconstructions of the genus *Symbiodinium* inferred using maximum likelihood analysis of (A) nuclear large subunit ribosomal DNA (nr28S-rDNA) and (B) chloroplast large subunit ribosomal DNA (cp23S-rDNA) (modified from Pochon and Gates, 2010). Next to each of the nine *Symbiodinium* clades (A–I) are the year each clade was first reported (left) and the year each was first recorded in hexacorals or octocorals (right). Discoveries marked in red indicate those that involved scuba to collect field samples. Question marks (?) indicate that *Symbiodinium* in clades E, H, and I have not been detected yet in corals.

clades has been shown to be spectacularly rich (e.g., LaJeunesse, 2002, 2005; Coffroth and Santos, 2005). A long-standing tenet in algal symbiosis holds that different invertebrate host species are specific to particular *Symbiodinium* (Trench, 1989, 1992), and that although a particular symbiont taxon might be found in a variety of diverse hosts, each host species uniquely associates with only one algal type. The use of scuba, combined with the development of PCR-based molecular methods, has allowed researchers to directly test this theory by obtaining snapshots of algal symbiont diversity in corals over time and space. These surveys have shown that while most corals indeed tend to be dominated by particular symbionts (LaJeunesse, 2001, 2002; LaJeunesse et al., 2004a, 2004b; Goulet, 2006; Goulet et al., 2008), many host species show a surprising degree of intraspecific symbiont diversity and exhibit considerable variability over time and space, with ontogenetic and environmental factors often driving observed specificity (Baker, 2003; Baker and Romanski, 2007). The use of scuba has been essential in this research by allowing coral species to be sampled from a variety of different sites, habitats, and depths. Coral species that routinely host multiple symbiont taxa (variously described as "flexible," "generalist," or "polymorphic" coral host species) have been distinguished from other species that have (to date) been found to contain only one particular symbiont type ("specific," "specialist," or "monomorphic" coral species). The reported diversity of symbionts from corals depends to a large degree on the breadth and depth of sampling effort, as well as the taxonomic and numeric resolution of the molecular methods used (Baker and Romanski, 2007). Scuba-based surveys of *Symbiodinium* diversity have therefore been a very active area of research for the last decade.

Rowan and Knowlton (1995) and Rowan et al. (1997) provide early examples of how scuba was employed to reveal these relationships. Their work showed that the dominant Caribbean corals *Montastraea annularis* and *M. faveolata* each contained *Symbiodinium* in three clades (A, B, and C), and that the distribution of clades in these coral species was determined by irradiance, both among colonies at different depths and within individual colonies. Moreover, during bleaching, symbionts in different clades varied in their sensitivity to high-temperature bleaching as a result of differences in their irradiance sensitivity. Together, the two studies convincingly showed that irradiance affects symbiont distribution over a coral landscape. The latter study also showed for the first time the important conclusion that symbiont genotype can directly influence fitness of the coral host.

Symbiodinium distributions also show strong biogeographic patterns. In scleractinian corals, perhaps the most obvious pattern is the contrast between the Atlantic and Indo-Pacific, with corals in the tropical western Atlantic (Caribbean) being codominated by *Symbiodinium* in clades A, B, and C but corals in the Indo-Pacific being dominated by clade C (Baker and Rowan, 1997; LaJeunesse, 2002). In both oceanic provinces, clade D is commonly found in environments characterized by chronic temperature stress (Chen et al., 2003; Baker et al., 2004; Fabricius et al., 2004) or in coral hosts that have recently experienced bleaching (Glynn et al., 2001; Baker et al., 2004; Jones et al., 2008; LaJeunesse et al., 2009a).

More recently, diversity assessments have focused on the identification of symbionts at finer taxonomic scales. LaJeunesse (2001) first formalized these investigations by proposing the Internal Transcribed Spacer 2 region of ribosomal DNA (rDNA) as a suitable marker for investigating subcladal diversity in *Symbiodinium*. This marker has since been extensively used to investigate diversity in samples of natural populations worldwide that were collected using scuba (e.g., LaJeunesse et al., 2010). Additional investigations of fine-scale diversity have used length heteroplasmy in chloroplast large subunit ribosomal DNA (rDNA) to survey symbiont diversity in Caribbean octocorals (Santos et al., 2004) and variation in microsatellite flanking sequences to further assess variation within these types (Santos et al., 2004). Allelic variation in microsatellite loci have been used to screen intraspecific variation in soft corals on the Great Barrier Reef (Howells et al., 2009) and octocorals in the Bahamas and the Florida Keys (Santos et al., 2003; Kirk et al., 2009), and to study reef endemism, stability, and fine-scale host specificity of symbionts in *Montastraea* spp. in the Florida Keys and the Bahamas (Thornhill et al., 2009).

The findings from these studies have revealed that *Symbiodinium* is extraordinary, not just for its taxonomic breadth (nine clades, each of which might ordinarily be considered a genus in its own right), but also for the taxonomic richness within many of these clades, with some (such as clade C) potentially containing dozens of distinct taxa (species), each of which is characterized by additional intraspecific variation. Some debate exists over the functional role of this *Symbiodinium* genetic diversity (Van Oppen and Gates, 2006; Apprill and Gates, 2007), but this has not prevented several new species of *Symbiodinium* from being introduced informally into the literature even though from a genetic standpoint they differ very little (e.g., LaJeunesse et al., 2009b). Drawing generalized conclusions from this sometimes bewildering level of diversity has proved difficult, as evidenced by the fact that no review has yet attempted to reconcile the data from the many dozens of papers published over the last decade. It seems clear that some degree of taxonomic revision is required when one compares diversity within *Symbiodinium* to that found in other groups (Stern et al., 2010), yet there is surprisingly little consensus on how to proceed.

Stability in Coral–Algal Symbiosis through Time

One area where scuba has contributed significantly to our understanding of coral–algal symbiosis is the degree to which the composition of symbiont assemblages in individual coral colonies can change over time. Of particular interest are the role of the environment in controlling potential changes and the importance of human-mediated disturbance, including global climate change, in determining the rate at which change might occur. These ideas were first introduced as the adaptive bleaching hypothesis (Buddemeier and Fautin, 1993). This hypothesis posited

that recovery from bleaching provides an opportunity for corals to become populated with different symbionts, and that changes in symbionts might prove beneficial to the coral host (i.e., have adaptive value). These ideas were further refined (Ware et al., 1996) and clarified to include quantitative changes in mixed symbiont assemblages as a result of bleaching (Baker, 2003; Buddemeier et al., 2004; Fautin and Buddemeier, 2004).

Numerous studies have tested this hypothesis and found evidence both in support of it (Baker, 2001; Kinzie et al., 2001; Berkelmans and van Oppen, 2006; Jones et al., 2008) and against it (Goulet and Coffroth, 2003a, 2003b; Rodriguez-Lanetty and Hoegh-Guldberg, 2003; Iglesias-Prieto et al., 2004; LaJeunesse et al., 2004a, 2004b; Kirk et al., 2005; LaJeunesse, 2005; Hannes et al., 2009). Although a consensus has not yet been reached, the combined evidence suggests that changes in symbiont assemblages that occur following bleaching are the result of changes in the relative abundance of preexisting symbionts, not the result of the acquisition of symbionts from external sources during bleaching or initial recovery (Baker, 2004). Although adult corals have been shown to be capable of acquiring symbionts from the environment (Lewis and Coffroth, 2004), these symbionts may be transient (Coffroth et al., 2010) and might not be able to achieve dominance within colonies regardless of environmental conditions or disturbance regime. Consequently, the extent to which bleaching can lead to new symbiotic combinations hypothetically depends largely on which symbionts are already present in the symbiotic assemblage of different coral species. In this context, attempts to identify and quantify background symbionts using real-time PCR are essential in determining whether corals routinely host a variety of symbionts at low abundance, or whether most coral species exclusively host specific symbiont types. An improved understanding of corals' specificity and the molecular, environmental, and ontogenetic factors that drive this specificity will allow us to critically assess the potential for adaptive bleaching in response to changing environmental conditions.

Ontogeny of Coral–Algal Symbiosis

The tremendous dinoflagellate diversity that exists among and often within host species is first established during the early ontogeny of the coral–algal symbiosis. Although in some cnidarian symbioses the symbiont is passed from parent to offspring (vertical or closed symbiont transmission), among the majority of corals, especially corals that free-spawn eggs and sperm, the offspring lack symbionts (aposymbiotic) and must obtain them anew each generation from the surrounding environment (horizontal or open transmission; Stat et al., 2006). Laboratory studies have demonstrated that in corals and other cnidarians initial uptake is relatively nonspecific and a range of different symbiont types can be acquired (Schwarz et al., 1999; Coffroth et al., 2001; Weis et al., 2001; Rodriguez-Lanetty et al., 2004; Poland, 2010). For example, *Montastraea faveolata* and *Acropora palmata* larvae acquired nine of eleven *Symbiodinium* types offered (Table 1) although only a subset of these types are dominant in the adult holobiont. These studies generally demonstrate that although juvenile corals can take up a broad range of potential symbionts early in ontogeny, not all symbionts are ultimately incorporated (or acceptable) to the coral species as adult colonies. These findings are similar to other laboratory studies that show that while newly settled corals and other cnidarians can harbor a diverse range of symbiont types, there is a degree of selectivity within the coral host such that not all types offered are taken up and even fewer are able to establish and sustain the symbiosis (Coffroth et al., 2001; Weis et al., 2001; Rodriguez-Lanetty et al., 2006; Mieog et al., 2009; Voolstra et al., 2009b).

TABLE 1. Different strains of *Symbiodinium* used to infect larvae of *Montastraea faveolata* (6d) and *Acropora palmata* (8d). Strain nomenclature is based on sequence variation in the 23S rDNA gene. Symbols: (-) indicates no infection observed; (+) and (++) indicate intensity of infection observed.

Culture	Strain	*M. fav.*	*A. pal.*
Control	None	-	-
ELI	A198	-	-
KB8	A194	++	++
04-503	A194	++	++
Acp343	B184	-	-
Mf1.05b	B184	++	++
Mf1.05b.01	B184	++	++
Mf10.14b.02	B224	++	++
Mf11.05b.01	B224	++	++
Mf6.07B	F178	+	+
Mf8.3T	F178	++	+
Mf10.08	D206	++	++

While these laboratory studies are informative of the potential symbiont diversity within cnidarian symbioses, in nature the symbiont pool is more diverse. Field studies enabled by scuba of initial host infection have shown that the developing coral acquires a wide assortment of *Symbiodinium* by accepting not only multiple strains from the same clade, but also multiple clades. This diversity is recorded both within and among juveniles (Coffroth et al., 2001; Little et al., 2004; Coffroth et al., 2006; Thornhill et al., 2006; del C. Gómez-Cabrera et al., 2008; Abrego et al., 2009a, 2009b; Mieog et al., 2009; Poland et al., 2013. For example, using scuba to deploy recruits to the reef and then monitor symbiont uptake over time, Poland et al. (2013) found between five and nine symbiont types among newly settled octocoral recruits (*Briareum asbestinum*) at any site or during any year (symbiont richness). Within the individual recruits, however, the majority hosted one or two symbiont types simultaneously. Fewer recruits (0.2% or less) harbored five or six symbionts simultaneously, even when the total number of symbiont types found across all juveniles at a particular site and/or year was

higher (i.e., the diversity of available symbionts was higher than *in hospite* diversity within a single juvenile (Poland et al., 2013). This leads to a symbiont complement within and among newly settled recruits that is more diverse than that within the adult holobiont (Coffroth et al., 2001; Little et al., 2004; Coffroth et al., 2006; del C. Gómez-Cabrera et al., 2008; Abrego et al., 2009a, 2009b; Thornhill et al., 2009).

Many studies confirm that the symbiont type initially acquired by a host is often not the symbiont type that predominates in the adult symbiosis. Some host species may acquire the symbiont types found within the adult along with other symbiont types (Coffroth et al., 2001; Weis et al., 2001; del C. Gómez-Cabrera et al., 2008), while in other host species the symbiont type that predominates in the adult symbiosis is not detected initially in symbiont assemblages of the juveniles (Little et al., 2004; Thornhill et al., 2006; Abrego et al., 2009a; Poland, 2010; Poland et al., 2013). For example, in a study utilizing clade-level analysis (del C. Gómez-Cabrera et al., 2008), newly settled recruits (10d) of *Acropora longicyathus* harbored mainly clade A *Symbiodinium* although clade C *Symbiodinium* dominated the adult symbiosis. After 83d, the proportion of *Symbiodinium* clade A decreased, *Symbiodinium* type C increased, and *Symbiodinium* type D was also observed. Similar observations have been reported for other acroporids (*A. tenuis* and *A. millepora*) where juveniles quickly acquired *Symbiodinium* within clade D although adults of these species predominantly harbor *Symbiodinium* types within clade C (Little et al., 2004; Abrego et al., 2009a, 2009b). Contrasting symbiont diversity among juveniles (higher) versus adult (lower) hosts is also seen in other groups such as octocorals, scyphozoans, and tridacnid clams (Coffroth et al., 2001; Belda-Baillie et al., 2002; Thornhill et al., 2009). In each of these studies, the use of scuba enabled the high-resolution sampling of the adult colonies, the detailed placement of new recruits at different sites, and the careful monitoring of symbiont uptake. These studies imply that over time (hours to years) a winnowing process occurs (sensu Nyholm and McFall-Ngai, 2004), so that only one to a few types establish and sustain the long-term symbioses found in the adults (Coffroth et al., 2001; Weis et al., 2001; Belda-Baille et al., 2002; Little et al., 2004). In some species this winnowing process involves large-scale, clade-level changes (e.g., Abrego et al., 2009a, 2009b) whereas among other groups the change is seen at the subcladal level (e.g., Poland, 2010). In some corals, the symbiont assemblage that is typical of an adult host colony does not become established until three to four years into the coral's ontogeny (Abrego et al., 2009b; Poland, 2010). It is not presently resolved at this time if this is the case in the majority of host species.

Future Directions

Scuba has enabled us to routinely sample symbiont diversity within important reef symbioses and to conduct careful in situ experiments to elucidate mechanisms that might be driving this diversity. However, we are still faced with many unanswered questions. Although numerous studies have contributed knowledge of processes involved in the initial infection and winnowing (e.g., Lin et al., 2000; Rodriguez-Lanetty et al., 2004; Wood-Charlson et al., 2006; Dunn and Weis, 2009; Voolstra et al., 2009a), the underlying processes and the ecological significance of initially accepting multiple types and then narrowing the assemblage to a single or a few types remains to be elucidated.

CORAL–MICROBIAL ASSOCIATIONS

Corals possess a high abundance and diversity of associated bacteria and archaea in their tissues, carbon-rich surface mucus layers, and skeletons (Ferrer and Szmant, 1988; Banin et al., 2000; Frias-Lopez et al., 2002; Rosenberg, 2007; Rosenberg et al., 2007; Shnit-Orland and Kushmaro, 2009). The diversity of coral-associated microbes has now been reasonably well documented using a variety of culture-independent gene surveys of microbial diversity (Rohwer et al., 2002; Bourne and Munn, 2005; Wegley et al., 2007; Thurber et al., 2009; Sunagawa et al., 2010). These surveys indicate that it is typical for a single coral to house many of the known divisions of bacteria. We now recognize this diversity, yet the functional roles and influences (positive, negative, or neutral) of the different microbes within these diverse coral–microbial assemblages are still poorly understood.

There are clear examples of microbes negatively impacting the coral host, most notably the pathogenic microbes associated with more than twenty documented coral diseases (Sutherland et al., 2004; Rosenberg et al., 2007; Bourne et al., 2009) and the case of *Vibrio*-induced coral bleaching (Kushmaro et al., 1996; Rosenberg et al., 2007). There are also clear examples of positive impacts of coral-associated microbes (Mouchka et al., 2010), including their roles in nutrient cycling (Rohwer et al., 2002; Lesser et al., 2004; Wegley et al., 2004; Beman et al., 2007; Wegley et al., 2007) and the production of antimicrobial compounds (Kelman et al., 1998; Ritchie, 2006; Nissimov et al., 2009; Mao-Jones et al., 2010; Rypien et al., 2010). In many cases, however, we still do not have a good picture of what natural, versus perturbed, coral–microbial assemblages look like, and it has been difficult to document clear species-specific associations between microbes and their coral host, including symbiotic microbial associations. Despite these knowledge gaps, coral–microbiological research has made great strides over the last 20–30 years. Here we focus on two recent advances in coral–microbial research: (1) increased knowledge of the ontogeny of coral–microbe associations and (2) the use of coral metagenomics to characterize microbial diversity and function. Both promise to transform our understanding of coral–microbial interactions.

The Ontogeny of Coral–Microbe Associations

While our understanding of the nature of the coral–algal symbiosis has improved greatly in recent years, only recently has research focused on the ontogeny of coral–microbial associations.

Pioneering work on this subject comes from two studies (Apprill et al., 2009; Sharp et al., 2010) wherein the authors followed the establishment of the microbial assemblage through the early stages of larval development in multiple broadcast-spawning corals. The first study, by Apprill et al. (2009), examined the ontogeny of microbial associations in the Hawaiian coral *Pocillopora meandrina*, a broadcast-spawning coral that vertically transmits its algal symbionts by seeding its eggs with *Symbiodinium* cells. Apprill et al. (2009) found that unlike vertically transmitted algal symbionts, bacteria are not taken up vertically but instead are acquired horizontally from the environment by the planula larvae after approximately 79 hours in the water column. Interestingly, they discovered that a clade of *Roseobacteria* in the genus *Jannaschia* consistently associated with the coral planula larvae. *Roseobacteria* are known to form associations with both phytoplankton and *Symbiodinium* (Littman et al., 2009b; Littman et al., 2010). This suggests that they might be associated with *Symbiodinium* inside the coral host, and yet if that is the case it is not clear why they would not be transmitted vertically with the *Symbiodinium* in *Pocillopora* eggs.

The second study, by Sharp et al. (2010), examined the ontogeny of microbes associated with seven species of broadcast-spawning corals (both Pacific and Caribbean species) that do not vertically transmit *Symbiodinium* in their eggs. By following the development of bacterial associations from the coral gametes through the swimming planulae to the newly settled polyps by using fluorescence in situ hybridization (FISH) techniques, Sharp et al. (2010) showed that microbes were only prevalent in the corals in the settled polyp stage (i.e., postsettlement), rather than established in the planula larvae as observed by Apprill et al. (2009) in *Pocillopora*. Taken together, these early results indicate that the ontogeny of these microbial associations differ in their timing depending on the coral. Nothing is known yet about microbial transmission in coral species that brood larvae, where vertical transmission of *Symbiodinium* is the most common mode.

Work by Littman also sheds light on how microbial assemblages change during ontogeny. Newly settled corals have a far more diverse microbial assemblage than older recruits, which are characterized by relatively more predictable, lower-variance assemblages (Littman et al., 2009b). This suggests a winnowing process whereby the more diverse microbial assemblage of juveniles is gradually replaced by the more characteristic adult microbial assemblage, much like what is seen with *Symbiodinium*. This ontogenetic pattern coupled with persistent variation in composition among sites (Littman et al., 2009b) provides a large role for environment in determining final composition.

Coral Metagenomics

Next-generation sequencing techniques are providing unprecedented access to and information about the genetic diversity of coral-associated microbes. Culture-independent genetic profiles of coral–microbial assemblages have become a mainstay of coral microbiology and are the primary tool used to examine the diversity, abundance, and associations of microbes on corals. The first genetic surveys relied heavily on Sanger sequencing bacterial 16s rDNA diversity from coral microbe clone libraries. Sequencing 16s rDNA clone libraries from corals revealed the high microbial diversity on corals (Rohwer et al., 2002; Bourne and Munn, 2005; Pantos and Bythell, 2006; Sunagawa et al., 2009), but the depth of sequencing has typically been limited to tens to hundreds of sequences per coral sample because of the high cost. Recent advances in high-throughput sequencing techniques now allow us to profile coral microbial diversity across hundreds of thousands, even millions, of sequences (Sunagawa et al., 2010) using either a target gene approach, like 16s rDNA sequencing (Sunagawa et al., 2009, 2010), or a metagenomic approach where the DNA or RNA content of an entire sample is shotgun sequenced, assembled, and annotated by bacterial group and gene function (Dinsdale et al., 2008a; Vega Thurber et al., 2008, 2009).

Deep-sequencing profiles of microbial 16s rDNA diversity from seven Caribbean corals (Sunagawa et al., 2010) uncovered even greater levels of novel coral-associated microbial diversity than had been seen with traditional Sanger 16s rDNA sequencing efforts. The Sunagawa et al. (2010) study also indicates that each coral species harbors an unprecedented level of endemic microbial diversity, toppling prior estimates of diversity in coral reef ecosystems (Sunagawa et al., 2009, 2010). While there was an overlapping of the microbial lineages from the adjacent water column and those from the sampled coral species, the large number of microbial taxa that were present on each coral species suggests that coral research will continue to contribute newly discovered microbes to science (Sogin et al., 2006; Pedros-Alio, 2007). These findings add an important microbial diversity–based perspective to the significance of conserving coral reefs.

Recent metagenomic approaches applied to corals demonstrate that it is possible to simultaneously profile coral microbes with rDNA sequences and categorize and annotate functional genes (Wegley et al., 2007; Dinsdale et al., 2008a, 2008b; Marhaver et al., 2008; Vega Thurber et al., 2008, 2009). Wegley et al. (2007) first demonstrated that metagenomics could be used to successfully profile the coral microbiome—including the algal, fungal, bacterial, and viral components—as well as to characterize the responses of members of the coral holobiont based on the function of particular gene sequences. The Wegley et al. (2007) study documented the high abundance of viral phages on corals plus an underappreciated role of fungi in nitrogen fixation.

Thurber et al. (2009) extended this coral metagenomic approach and profiled the changes in coral–microbial assemblages on *Porites compressa* associated with four important coral stressors: increased temperature, increased nutrients, increased dissolved organic carbon, and higher acidity (i.e., lower pH). They observed strong shifts in the microbial assemblages between healthy and stressed corals, as well as shifts to genes involved in virulence and stress resistance due to coral stress. Interestingly, their results indicate that *Vibrios* caused strong shifts in

the microbiome metabolic profiles during the temperature-stress treatment.

Future Research Directions for Coral–Microbial Interactions

New insights into the ontogeny of coral–microbial interactions and metagenomic approaches are allowing us to characterize the onset and dynamics of coral–microbial assemblages with more depth than ever before. Significant questions remain about the specific roles of particular microbial groups in corals. For instance, what is the role of the microbial assemblage in host fitness? How flexible is the partnership between corals and their microbial assemblage in coping with climate change? How flexible is the partnership between the a coral host's *Symbiodinium* of choice and its algal-associated microbes? If the coral–algal symbiosis is being threatened by chronic stress, are there microbial-antagonistic effects driven by the holobiont or are opportunistic microbes driving the holobiont physiology?

EMERGING ROLE OF THE CORAL HOST

Although great strides have been made that increase our understanding of the importance and roles of *Symbiodinium* and microbial diversity in corals, we know far less concerning the role of the coral host in regulating and maintaining this diversity. This lack of knowledge about the role of the coral host has in some cases led to bias in favor of the importance of algae or microbes in the relationship (Baird et al., 2009). For example, studies of *Symbiodinium* diversity and flexibility have suggested that changes in algal symbiont assemblages will help corals survive environmental change but have tended to downplay how coral specificity might limit this process (Baker, 2001; Baker et al., 2004; Berkelmans and van Oppen, 2006; Jones et al., 2008). Similarly, with coral–microbe interactions, the probiotic hypothesis championed by some coral microbiologists proposes that microbes regulating microbes act as the de facto coral immune system (Ben-Haim et al., 2003; Rosenberg et al., 2007a, 2007b) even though there is clear evidence that coral have innate immune systems as well (Mydlarz et al., 2006, 2010; Miller et al., 2007; Dunn, 2009). Yet new data from coral genomics and transcriptomics are providing novel insights into the genetic mechanisms controlling the relationship between the coral host and its algal symbionts, and into the nature of coral immunity.

The Role of Coral Genomics

Recent advances in coral genomics and transcriptomics are elevating our understanding of the role that the coral host plays in maintaining the stability of the coral holobiont. Gene expression analyses using microarrays have examined the response of the coral host to a variety of environmental stimuli during early ontogeny (Grasso et al., 2008; Reyes-Bermudez et al., 2009; Voolstra et al., 2009a; Polato et al., 2010; Portune et al., 2010) as well as in adult colonies (DeSalvo et al., 2008; Reyes-Bermudez et al., 2009; DeSalvo et al., 2010a, 2010b). Transcriptome profiles of larvae exposed to different choices of *Symbiodinium* strains were correlated with the profiles of unsuccessfully infected larvae, and the profiles of control larvae were correlated with those of successfully infected larvae (Voolstra et al., 2009b), suggesting that successful *Symbiodinium* strains enter the host in a stealth manner rather than triggering a cellular response (Voolstra et al., 2009b). In the case of adult symbioses under slight stress (e.g., thermal), particularly in the coral *Montastraea faveolata*, which harbors multiple strains at once, the transcriptome response seems to be driven mainly by the algal complement (DeSalvo et al., 2010a). In contrast, when the stress is severe the transcriptome profiles indicate clear cellular responses driven by the host coral (DeSalvo et al., 2008).

Recently there has been an explosion of next-generation sequencing that is expanding this initial set of coral and algal transcriptomes, but few are published (Meyer et al., 2009; Meyer and Matz, 2010). With costs dropping and high-throughput capacity increasing exponentially, de novo whole-genome shotgun sequencing is now within reach for coral reef science. Several coral, *Symbiodinium*, and microbial genome projects are expected to come online in the near future. Once host, algal, and microbe genomes are complete, and as transcriptome sequencing becomes more commonplace, our ability to move coral research to a systems biology level will be greatly enhanced and new "-omic" technologies can be brought into the study of the coral holobiont.

Coral Immunity

Recent progress also has been made in understanding the coral immune response in fighting off disease (reviewed by Mydlarz et al., 2006, 2010; Miller et al., 2007; Dunn, 2009) as well as in the coral–algal symbiosis (Weis, 2008; Weis et al., 2008; Weis and Allemand, 2009). Like other invertebrates, corals have innate immune systems capable of self-/non-self-recognition (Hildemann et al., 1975; Neigel and Avise, 1983) and the ability to identify and react to pathogen infection (Mydlarz and Harvell, 2007; Mydlarz et al., 2008, 2009, 2010). Recent genetic surveys demonstrate that corals and their anthozoan relatives possess a relatively full set of the genes and gene pathways involved in innate immunity (Miller et al., 2007; Dunn, 2009; Mydlarz et al., 2010), including the three major innate immune pathways: the Toll-like receptor (TLR) pathway (Miller et al., 2007), the Lectin Complement pathway (Miller et al., 2007; Kvennefors et al., 2010), and the Prophenoloxidase (PPO) pathway (Mydlarz et al., 2008). Corals lack adaptive immunity (i.e., immune specificity and memory), which is restricted to jawed vertebrates.

To date, most of the information that we know about the immune responses of corals (hard and soft) comes from histological and biochemical data focused on specific immune assays from a few coral species (Mydlarz et al., 2010). Histological data

suggest that mobile amoebocytes, which move between the coral ectoderm and endoderm in the mesoglea, act like immune cells and aggregate at regions where tissues are damaged (Mydlarz et al., 2008, 2009; Palmer et al., 2008). For example, Mydlarz et al. (2008) documented aggregations of these mobile amoebocytes in the sea fan *Gorgonia ventalina* at the site of infection by pathogenic *Aspergillus sydowii* fungus. Likewise, in the reef coral *Acropora millepora* Palmer et al. (2008) documented amoebocyte aggregations associated with inflammation and melanization in abnormally pigmented coral tissues. Other studies reveal potential for antioxidant activity associated with coral fluorescent proteins (Palmer et al., 2009). Recent work increases the taxonomic range of comparison to 10 coral families and shows links between susceptibility to bleaching and disease and sizes of melanin granules, levels of PPO activity, and fluorescent proteins (Palmer et al., 2010).

At the biochemical or genetic level, characterizations of the coral immune response thus far have focused primarily on the Prophenoloxidase (PPO) pathway using biochemical assays of enzymatic activity. Prophenoloxidase immune response acts via the PPO pathway, which causes pathogens to be targeted, encapsulated in melanin, and ultimately degraded by phagocytosis (Mydlarz et al., 2006, 2008, 2010). Increased PPO activity and melanization has been detected in *Aspergillus*-infected sea fans (Mydlarz and Harvell, 2007; Mydlarz et al., 2008), as well as in the pigment anomalies in the reef coral *A. millepora* (Palmer et al., 2008) and in bleached *Montastraea faveolata* corals (Mydlarz et al., 2009). These data suggest that melanization and degradation by the PPO pathway is an important innate immune response in both soft and hard corals. No one has yet profiled the immune response of an infected coral using gene-expression approaches across the full range of possible immune pathways, and thus it is not yet known what other pathways might be important in coral immunity in general.

Weis and colleagues (Weis, 2008; Weis et al., 2008) have begun to focus on the potential links between the coral innate immune response and the relationship between the coral host and its symbiotic algae. According to the current hypotheses, one key to the maintenance of the coral–algal symbiosis is the ability of symbionts to modify the host's immune response. Algal symbionts are contained in specialized vacuoles in the coral endoderm and are acquired through a process similar to phagocytosis of pathogens. Early data suggest that the acquisition of the symbionts is mediated by pattern recognition receptors (PRR) (Weis et al., 2008), such as lectins (Wood-Charlson et al., 2006; Kvennefors et al., 2010), that are down-regulated during the early ontogeny of the coral–algal symbiosis (Wood-Charlson et al., 2006). Phagocytosis also appears to be arrested during symbiont acquisition (Chen et al., 2005; Schwarz et al., 2008) and reactivated when nonspecific symbiont types enter the host cells (Dunn and Weis, 2009). These preliminary data suggest that there is a strong and important link between the coral immune system and the evolution of the coral–algal symbiosis that warrants further investigation.

Future Directions

These recent studies demonstrate that the coral host has a viable innate immune system and can respond to pathogen infection. Genetic data examining the relationship between the coral host and its symbionts also indicate clear links between the innate immune response of the coral and the mechanisms by which algal symbionts become established within their hosts. These early findings are just beginning to elucidate how the coral immune system operates and how symbionts evade or modify the host's immune response during uptake. Many questions remain unanswered. For example, does the innate immune response show specificity according to the type of pathogens (i.e., viral, bacterial, or fungal)? If so, what innate immune pathways are involved? Similarly, if *Symbiodinium* modifies the immune response during uptake, what genes or gene pathways are also modified and how does this impact coral innate immunity? In the future, experimental work combining infection experiments and genetics promises to answer these questions about the nature of coral innate immunity and the role of the coral host in responding to pathogen and symbiont infection.

CONCLUSIONS

Great progress has been made in understanding the nature of the interactions within the coral holobiont since Rohwer et al. (2002) proposed the concept. We now have a much stronger understanding of the coral–algal symbiosis, including knowledge about its ontogeny, specificity, and flexibility. Knowledge about the nature of coral–microbe interactions is growing by leaps and bounds with increasing interest in coral microbiology and the incorporation of new (meta) genomic techniques to address questions about the makeup and dynamics of coral microbial assemblages. Knowledge about the role of the coral host is growing as we gain insights into the nature of coral innate immunity, including how pathogens are detected and how eukaryotic and/or microbial symbionts modify response or elude detection. As we continue to progress, the concept of the coral holobiont will remain important because the greatest strides in the field will be made by understanding how the complex sets of organisms making up the coral holobiont— the coral host and associated eukarya, bacteria, archaea, and viruses—interact and function both synergistically and sometimes antagonistically as a community or ecosystem.

ACKNOWLEDGMENTS

We thank Michael Lang, Phil Taylor, Roberta Marinelli, and Susan Roberts for organizing the symposium, the Smithsonian Institution and the National Science Foundation for providing funding and logistical support, and all our fellow participants. Additional funding was provided by National

Science Foundation grants to SVV (OCE-0848345), ACB (OCE-0099301 and 0527184), MAC (04-24994 and 09-26822), MAC and MM (03-13708), and CDH and LM (OCE-0849799). ACB was also supported by grants from the Lenfest Ocean Program, the Tiffany and Co. Foundation, and a Pew Fellowship in Marine Conservation.

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