Review

Microbial disease and the coral holobiont

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Tropical coral reefs harbour a reservoir of enormous biodiversity that is increasingly threatened by direct human activities and indirect global climate shifts. Emerging coral diseases are one serious threat implicated in extensive reef deterioration through disruption of the integrity of the coral holobiont – a complex symbiosis between the coral animal, endobiotic alga and an array of microorganisms. In this article, we review our current understanding of the role of microorganisms in coral health and disease, and highlight the pressing interdisciplinary research priorities required to elucidate the mechanisms of disease. We advocate an approach that applies knowledge gained from experiences in human and veterinary medicine, integrated into multidisciplinary studies that investigate the interactions between host, agent and environment of a given coral disease. These approaches include robust and precise disease diagnosis, standardised ecological methods and application of rapidly developing DNA, RNA and protein technologies, alongside established histological, microbial ecology and ecological expertise. Such approaches will allow a better understanding of the causes of coral mortality and coral reef declines and help assess potential management options to mitigate their effects in the longer term.

The importance of coral disease research

Loss of biodiversity is a significant and concerning issue across all ecosystems. Global wildlife extinction rates are estimated to be 100–1000 times higher than historical normal levels, and up to 50% of higher taxonomic groups are critically endangered [1]. Marine ecosystems are included in this dire assessment. Recent estimates report that one-third of all coral species are at risk of extinction [2]. Coral reefs are fundamental to the prosperity of many countries throughout the world: they support food production, tourism and emerging biotechnology development, and provide coastal protection from natural disasters. However, recent assessments [3] show that approximately 19% of coral reefs have been effectively destroyed with no immediate prospects of recovery. An additional 15% are under imminent risk of collapse through human pressures within the next 10–20 years, and a further 20% are under a longer-term threat of collapse.

Diseases have been identified as a major contributor to decline in corals worldwide (Figure 1), particularly in the Western Atlantic, where epizootics have led to significant losses of coral cover and shifts in community structure [4–6]. Whether these reported diseases are a result of new pathogens or changed environmental conditions is a current topic of debate [7]. The potential damage inflicted by disease to coral reefs is best exemplified by the observations in the Caribbean, where successive disease outbreaks caused a complete shift in the competitive dominant coral and contributed to an apparent ecological phase shift toward an algae-dominated ecosystem [4,6,8] (Figure 2). Specific examples include the outbreak of white band on *Acropora palmata* and *Acropora cervicornis* in the 1980s causing an estimated 95% reduction in colonies [9] and white pox disease in the Florida Keys that further reduced the cover of *A. palmata* by up to 70% [5]. Longer-term paleontological evidence suggests that the extent of recent losses in one coral species (*A. cervicornis*) as a result of disease is unprecedented on a time-scale of at least three millennia [4].

Given the global ecological and economic importance of tropical coral reef ecosystems, gaining an understanding of what is causing mortality on these reefs is a vital and

Glossary

Coral holobiont: a collective term referring to the totality of a coral animal, its endosymbiotic zooxanthellae, and the associated community of microorganisms including bacteria, archaea, viruses, fungi and endolithic algae.
Disease causation: factors leading to the onset of disease, including the agent(s), their virulence factors, mode of replication and transmission, plus host response and environmental cofactors.
Epizootic: a disease that appears as new cases in a given population, during a given period, at a rate that substantially exceeds what is expected based on recent experience (i.e. a sharp elevation in the incidence rate).
Lesion: any morphologic or biochemical anomaly in an organism.
Microbiome: the totality of microbes, their genetic elements (genomes) and environmental interactions in a defined environment.
Prophenoloxidase activated melanization response: an important immune response and defense system in many invertebrates, characterized by melanization (i.e. an increase in pigmentation) and controlled by the enzyme phenoloxidase (PO).
Virus-like particles (VLPs): non-infectious entities resembling the virus from which they are derived and consisting of proteins from the outer shell and surface of the virus, but lacking the nucleic acid required for replication.
Zooxanthellae: members of the phylum Dinoflagellata, with the most common genus being *Symbiodinium* and representing a variety of species that form symbiotic relationships with other marine organisms, particularly coral.

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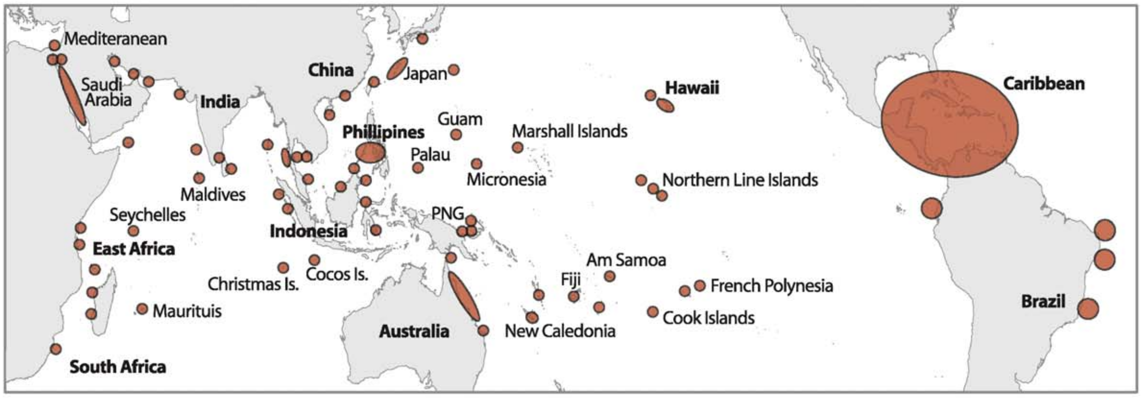

Figure 1. Worldwide reports of coral disease. Data derived from GCRMN database (http://www.unep-wcmc.org/) and literature search using coral and disease as descriptors. The red circles (and ellipses) represent the geographical area over which disease outbreaks have been reported and are not representative of disease effect.

pressing research focus. Coordinated research efforts to understand the mechanisms of these disease processes will allow development of strategies that effectively mitigate reef decline. In this article, we review current knowledge of microbial interactions causing coral disease and highlight the complexity of interactions between the host, the pathogen and the environment. We emphasize the importance of implementing multidisciplinary investigations that use both established and emerging technologies to clarify the fundamental factors leading to the observed increase in coral disease outbreaks. The coral holobiont represents an emerging model for studying invertebrate-microbial interactions and how climate drivers can disrupt the homeostasis of the holobiont, resulting in disease.

Current knowledge and research directions

Approximately 18 coral diseases have been identified thus far [7,8,10] (Table 1). Characterization of many of them has depended on field surveys, although pathological signs are so general that assessment of gross lesion morphology cannot differentiate between the diseases. This has resulted in confusion, with several diseases mistaken for others previously shown to be caused by a specific pathogen. Comprehensive case definitions systematically characterizing diseases at the gross, microscopic, immunologic and microbial level are therefore few and far between [11]. The dearth of knowledge about the actual causes and pathogenesis for many of these diseases is largely the result of too few researchers studying the interactions between the putative causative agents and the coral host, limitations in funding, and a complex host for which a basic understanding of physiology is still lacking. However, the field has had some notable success stories in spite of these challenges. Well characterized diseases include *Aspergillus sydowii* infection of gorgonians [12], the coral bleaching in *Oculina patagonica*

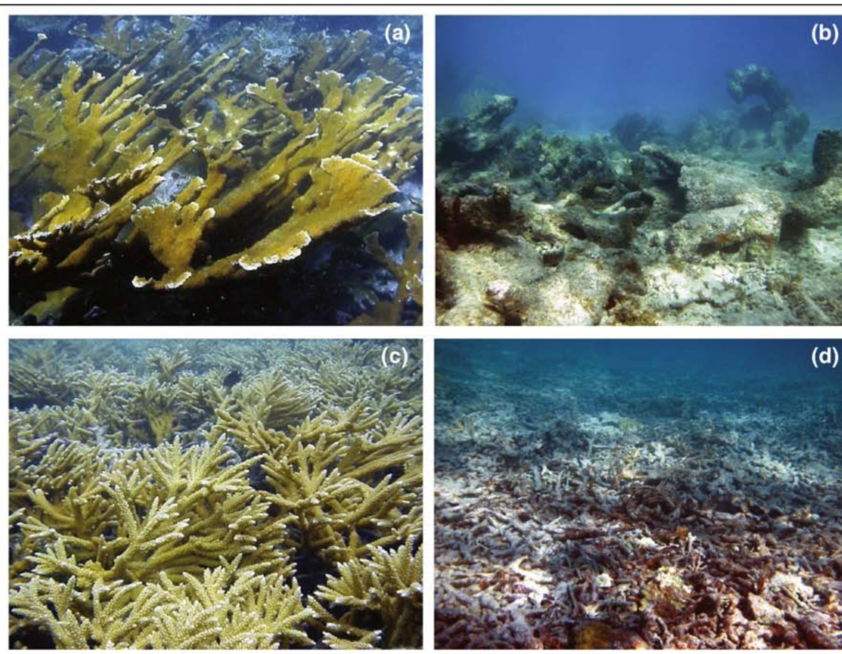

Figure 2. Time series photographs showing the recent demise of *Acropora* corals in the Caribbean. **(a)** Healthy *Acropora palmata* in San Cristobal in 1999 and **(b)** in 2009; **(c)** *Acropora cervicornis* and *Acropora prolifera* in San Cristobal in 2001 and **(d)** in 2009. Photo credits: Ernesto Weil.

Table 1. A historical comparison of human and coral infectious diseases

Category	Human	Coral
Number of host species	1	845 [2]
Number of described disease lesions	>1000[a]	18 [8]
Number of identified agents causing infectious and parasitic diseases	171[a]	6
Bacterial diseases	66[a]	4 [11]
Viral diseases	43[a]	0 [11]
Fungal diseases	15[a]	1 [11]
Parasitic diseases	47[a]	1 [75]
Number of years investigated	>2500 [45]	13 [11]
First pathogen identified	1854 [48]	1996 [76]
Number of researchers	>1 million[b]	~3500[c]

[a]World Health Organizations International Classification of Disease (ICD-10 codes; http://www.who.int/classifications/apps/icd/icd10online/)
[b]Paris: Institute of Statistics, UNESCO; 2004 (http://www.uis.unesco.org) and World Health Organization Global Health Atlas (http://www.who.int/globalatlas).
[c]Participants at the 2008 International Society for Coral Reef Conference (http://www.nova.edu/ncri/11icrs/index.html). This number represents participants at the 2008 conference working on aspects of coral reef environments. Researchers specifically working on aspects related to coral disease could be estimated to be less than 100.

caused by Vibrio shiloi [13] and black band disease (BBD) associated with a consortia of organisms that act together to kill coral tissue (Box 1). As with all animals, diseases of corals are the result of an interaction between host, agent and environment. Each of these factors poses its own unique challenges and research needs.

A complex host: a holobiont

Similar to other animals, a coral is a complex holobiont consisting of the animal and its associated suite of internal and external microbiota [11,14]. However, unlike most other animals, corals also possess symbiotic algae that reside intracellularly and provide crucial nutrients [15] and whose loss from coral tissues can lead to bleaching and death [16]. Other members of the holobiont include bacteria, archaea, viruses, fungi and endolithic algae, which also provide mutualistic benefits [14,17,18]. Disturbance or shifts in any of these partners can compromise the health of the whole animal. Disease onset is often a complex set of interactions among a variety of associated partners that affect the fitness of the collective holobiont. Thus, to understand disease within corals, an in-depth knowledge of the basic biology of each holobiont member is required. One current research focus is to elucidate the microbiota of healthy corals [14,19,20], which will aid our understanding of this multispecies mutualism and will help to identify which species play a key role in maintaining coral health. The interactions between holobiont microbial partners are also of interest because commensal relationships in multispecies associations determine the spatial distribution of the microbial populations. For example, antimicrobial properties in coral mucus select and structure the overall coral-associated microbial communities [18]. Other compounds such as dimethylsulfoniopropionate (DMSP) produced in high concentrations by dinoflagellates (including coral symbionts) probably provide nutrient sources for coral-associated bacteria and structure bacterial communities in corals, with important consequences for coral health [21].

In other animal diseases, an extensive knowledge of immune responses has been invaluable in understanding pathogenesis. However, our current understanding of coral immunology is rudimentary [22], particularly with respect to defences against infectious agents. Progress has been made in identifying key innate immune pathways associated with cellular immunity, the prophenoloxidase-activated melanization response [23,24] and basic oxidative pathways [23]. Enormous potential exists in using these measures to assess variation in coral resistance in the field [22–25], but many basic immunological questions remain with respect to the coral animal. The field would also benefit from standardized animal models for investigating coral disease [26], coral cell lines and commercially available reagents to study the genomes and proteins of the coral host. Considering all of these challenges, it becomes apparent that there are many opportunities for developing fundamental knowledge on coral physiology, immunology and cellular pathology.

Box 1. The BBD conundrum.

The oldest and perhaps most intensively studied coral disease to date, black band disease (BBD), provides an interesting example of the difficulties in elucidating and understanding the pathway of disease causation. Characterised by a thick microbial mat, it can migrate up to 2 cm per day across apparently healthy coral colonies, actively killing tissue and leaving exposed skeleton behind [63]. Extensive molecular-based diversity studies identified a range of microorganisms present within the disease band, including cyanobacteria [64–66], sulfate-reducing *Desulfovibrio* spp. [64,67], sulfide-oxidizing *Beggiatoa* spp. [68] and other heterotrophic microbes [64,66,68]. This complex structured and synergistic microbial community has resulted in difficulties in identification of the primary causative agent, although it hints at this disease being polymicrobial. Recent work by Richardson and colleagues has identified the synergistic action of an anoxic microenvironment, sulfide and cyanobacterial toxins all contributing to coral tissue degeneration [69,70]. Despite close to 40 years of research, the conditions that lead to the onset of lesions and causes of the disease are still unknown. Specific transmission modes, mechanisms of band formation and factors leading to the initiation of lesions on host corals are still poorly understood. Environmental factors such as light, nutrients and temperature have been correlated with prevalence and progression of disease, although their exact mechanisms are difficult to elucidate [71,72]. The challenges faced with the study of black band disease are consistent across all studies of corals and their lesions.

Microbes as causative agents of disease

Research has focused on characterizing and isolating microbiota, in particular bacteria, associated with coral lesions (Table 2). Many bacteria have been associated with gross lesions in corals, but confirming whether they actually cause the lesion has been difficult. Characterization of the host response to these microorganisms at the cellular level has often been lacking. Even in the few notable cases – BBD (Box 1), *Vibrio*-associated bleaching and aspergillosis [12,13] – where the interactions between the host and the agent have been well characterized at the gross, microscopic, molecular and biochemical level, shifts in agent virulence appear to be occurring that raise the question of whether or not initially isolated agents are still primary pathogens. For example, *Aspergillus sydowii*, identified as the primary microbial agent of the gorgonian epizootic from 1994 to 1997 [12], began to wane in 1998 [27] and only scattered cases currently appear in certain locations.

Table 2. A comparison of our understanding of human and coral disease causation[a]

Infectious process and ecology	Cholera [48]	*Vibrio shiloi* bleaching [13]	Aspergillosis in the Caribbean [12]	Bleaching and lysis in Red Sea and Indo-Pacific (white syndrome) [77,78]	White band in the Caribbean [4,79]	White plague in the Red Sea [80]	White pox in the Caribbean [5]	Yellow blotch in the Caribbean [81]	Black band (widespread, see Box 1)
Causative agent	√	√	√	+/−[b]	+/−[b]	+/−[b]	+/−[b]	+/−[b]	+/−
Mode of pathogen entry into host	√	√	−	−	−	−	−	−	+/−
Multiplication in tissues	√	√	−	−	−	−	−	−	−
Pathogen resistance to host defences	√	√	+/−	+/−	−	−	−	−	−
Mode of host tissue damage	√	√	−	+/−	−	−	−	−	+/−
Genetic basis of virulence	√	√	+/−	+/−	−	−	−	−	+/−
Pathogen reservoir and/or vectors	√	√	+/−	−	−	−	−	−	−
Mode of pathogen transmission	√	√	+/−	−	−	−	−	−	−
Host immune response	√	−	√	−	−	−	−	−	−
Environmental co-factors	√	√	+/−	+/−	−	−	−	−	+/−
Diagnostic pathogen detection	√	−	−	−	−	−	−	−	−
Management of disease epizootic	√	−	−	−	−	−	−	−	−

[a]√, Well known; −, no information available; +/−, some information available but exact mechanisms still unclear.
[b]Agents associated with lesions have been identified and Koch's postulates satisfied, but host response to these microorganisms at the cellular level is not fully elucidated.

In addition, *A. sydowii* has been found recently in association with apparently healthy gorgonians [28]. Similarly, recent work has shown that *Vibrio shiloi* is no longer associated with bleaching of the Mediterranean coral *Oculina patagonica* [11], with other studies suggesting that bacterial involvement in coral bleaching is that of opportunistic colonization [29,30]. This lack of consistency is due in part to the crude endpoints (tissue loss or discoloration) that are employed to define coral diseases. Many different factors can result in tissue loss, discoloration or death of a coral. Such uncertainties highlight the need to understand the cellular interactions between the host and the agent in addition to the interactions between the pathogen and the other holobiont associates (e.g. bacteria, viruses, zooxanthallae). Because we have not yet uncovered the mechanisms of these interrelated ecological interactions, it is not surprising that we continue to observe dynamics in disease causation that confuse our understanding. Based on the range of infectious agents associated with other animals, corals are probably susceptible to a wide range of prokaryotic, eukaryotic and viral pathogens. These agents might attack the coral animal directly, or attack the zooxanthellae, the surface microbial symbionts, or any combination of these, and therefore development of improved diagnostic tools is required. For example, recent work suggests that viruses play an important beneficial role in the coral holobiont [31–33], though may also act as potential pathogens [32,34]. A current challenge is the development of tools to investigate viruses associated with corals (Box 2).

A changing environment

The role of the environment in coral disease epizootics has been a major focus of investigations because coral reefs are the ecosystem facing the most rapidly advancing threat from climate change [35–37]. Corals are sessile organisms and thus afford the advantage that individual animals can be tracked in the wild to monitor disease progression. Significant challenges in framing the scale, complexity

Box 2. The role of viruses in coral disease

Viruses are the most abundant biological entities on the planet and are responsible for structuring marine prokaryotic and eukaryotic planktonic communities; however, only relatively recently has this role in controlling global biogeochemical cycles been discovered. Recent studies have identified viruses and virus-like particles (VLPs) in seawater surrounding corals and in close association with corals and their endosymbiotic *Symbiodnium* partners (reviewed in [32]). Currently there are no known viral pathogens of corals. However, Vega Thurber and colleagues [34] used a combined metagenomics and real-time PCR approach to show that in *Porites compressa* corals, herpes-like viral sequences rapidly increased when corals were subjected to stress (temperature, pH and elevated nutrients). In addition, reports of growth anomalies (or tumours) of coral are common, with suggestions that viruses might be involved [73] because they are implicated in cellular proliferative disorders or neoplasms in many other organisms [74]. Research on viruses and their detrimental effect on coral is an emerging field.

Interestingly, viruses might also have a beneficial role in coral health by maintaining homeostasis of the holobiont. Bacteriophage lysis of proliferating populations might stabilize the coral-associated bacterial community with the ''kill the winner'' approach, where an increase in abundance of one dominant bacterial population increases its contact with phages, leading to significant increases in infection and subsequent lysis, which then control its abundance. The exploitation of bacteriophages also provides one useful management option to control the spread of infectious coral disease. Lytic phages targeted against coral pathogens such as *Vibrio coralliilyticus* and *Thalosomonas loyaeana* were shown to prevent transmission and subsequent infections of the disease in controlled experimental trials [31,33].

and natural variability of emerging coral diseases throughout the world's oceans include a lack of historical baseline data sets and a wide diversity of ecological factors that influence disease patterns on both regional and global scales [38]. Progress has been made to clarify the effects and drivers of coral disease on local and regional scales [10,39–41], and the field continues to refine the methods of coral disease assessment [7]. However, developing a meaningful understanding of the interactions between the environment, the agent and the host is still required. For example, environmental disturbances such as increased temperature or excess nutrients are linked to increase reports of coral diseases [40,42,43]. These stressors trigger physiological and biochemical responses in the coral animal [44] and cause shifts in the associated microbial communities [30]. Such interactions highlight how each factor in the disease triad is inextricably linked and why investigations of causation must be approached from multiple angles to elucidate the interplay of the host, the agent and the environment.

Case study comparisons of human and coral diseases

The study of coral disease is in its infancy compared with human disease investigations. The first report of a disease affecting corals was made in 1973 when Antonius observed BBD destroying coral colonies in the Caribbean (Box 1). By contrast, human disease has been investigated since the time of Hippocrates [45]. A direct comparison of case examples for human versus coral diseases highlights gaps in knowledge when it comes to the causation, epidemiology, transmission and pathogenicity of coral diseases (Table 2).

Many coral diseases are attributed to bacteria of the *Vibrio* genus, and therefore it is instructive to outline a well-characterized *Vibrio* species that is pathogenic to humans. Cholera provides an excellent example of a well-defined human case study where research has established an understanding of the host–pathogen interactions and also environmental cofactors that influence disease dynamics [46]. Sometimes a fatal disease, cholera is caused by infection of *V. cholerae* and is transmitted through contaminated water into the human gut where it expresses a toxin that causes body fluids to flow across the lining of the intestine. Naturally abundant in fresh, estuarine and marine waters, there are over 200 *V. cholerae* serogroups, although only two are known to cause epidemics [46,47]. Cholera remains a persistent global health problem despite extensive research since it was first reported in 1849 by M. Gabriel Pouchet, and later rediscovered and *Vibrio* demonstrated to be the causative element by Robert Koch in 1884 [48]. Cholera represents a good disease model for determining how human activities (including global warming) directly affect the causative bacterium's ecology, persistence and spread in aquatic environments [49]. This parallels current research goals for coral diseases because human activities and climate change have been implicated in the increased prevalence of these diseases [39,40], either through changing pathogenicity of causative agents or increased host susceptibility.

The best-studied example of coral microbial infectious disease is the *Oculina patagonica*–*Vibrio shiloi* model of coral bleaching in the Red Sea (reviewed in [13]). This model system nicely displayed the process of infection and effect on the host leading to bleaching, including (i) chemotaxis and adhesion to a β-galactoside receptor in the coral mucus, (ii) penetration into epidermal cells, (iii) differentiation into a viable but not culturable (VBNC) state, (iv) intracellular multiplication, (v) production of toxins that inhibit photosynthesis and (vi) production of superoxide dismutase (SOD) to protect the pathogen from oxidative stress. Importantly, temperature acted as an environmental cofactor, altering the virulence of the pathogen through expression of the bacterial toxin, SOD and adhesion. Research on this model system from 2003 onwards showed that *V. shiloi* was no longer found associated with bleached coral, and *O. patagonica* was hypothesised to have become resistant to the bacterial infection, perhaps through the adaptive potential and symbiont switching of the holobiont [11]. Differences between the disease characteristics before and after 2003 highlight the dynamic nature of pathogen–host interactions [50].

The *O. patagonica*–*V. shiloi* model has been instrumental in elucidating coral pathogen interactions and spawning a new branch of wildlife disease investigation. However, our understanding in almost all other coral disease cases of the factors involved in infection and disease causation is poor (Table 2). Even for the *O. patagonica*–*V. shiloi* model system, some very important knowledge gaps exist. For example, bleached corals are no longer consistently associated with infection by *V. shiloi*, suggesting either host resistance to infection or other potential causes of bleaching [29,50]. These gaps highlight important areas of further research including efforts to understand the host immune and adaptive responses, vital for elucidating coral disease causation and dynamics.

Direct comparison of coral disease causation studies to those of our human disease case study, *V. cholerae,* highlights research priorities regarding interactions between agent, host and environment (Table 2). For cholera, the pathogen, mode of infection, genetic basis of virulence, reservoirs, vectors and environmental cofactors are all well described and understood [47–49]. Means to rapidly diagnose, treat and prevent this disease in humans are now available as a result of the knowledge that has evolved over 150 years of research. By contrast, although some progress has been made for a few coral diseases, coral reefs are in desperate need of similar tools to allow prompt identification of causes of disease and implementation of measures to manage, prevent or mitigate outbreaks. Coral reef ecosystems might not survive another 150 years given current decline; therefore, rapid advance in this field must occur by drawing knowledge from established research fields (Box 3).

The road ahead for coral disease studies

As coral disease researchers, how do we close the gap in knowledge between human disease causation versus coral disease causation? The tools historically employed in human and veterinary medicine have been successful in identifying causation and establishing standardized case definitions of many diseases. This has allowed rapid

Box 3. Questions for future research

- **What are the functional roles of all members of the coral holobiont?** How are the coral–microbe interactions regulated in the healthy holobiont, and how do shifts in holobiont members lead to a transition into a disease state? Determination of the complete coral microbiome (similar to the ongoing human microbiome project) and its resilience to a changing environment might answer some of these questions.
- **What are the agent(s) causing the majority of currently unclassified coral disease lesions?** This is a continuing research priority that should specifically aim to link the causative agent(s) with the host response at the cellular level, providing systematic and comprehensive case definitions at the gross, microscopic, immunologic and microbial levels.
- **What effect does a changing environment (increased temperature, nutrient stress and ocean acidification among others) have on coral fitness, microbial associates, pathogenic mechanisms and coral disease dynamics?** Research implementing robust aquarium and field based experimental designs combined with comprehensive ecological, biomedical, microbiology and genetic methodologies are essential.
- **How does the coral immune system function?** We suggest that research should specifically target the underlying mechanisms employed by the coral host to combat disease onset.
- **What roles do organisms other than bacteria (e.g. endolithic fungi, protozoa and viruses) play in coral diseases?** The beneficial and detrimental roles of viruses within the coral holobiont can now begin to be understood with developing imaging and genetics based technologies being adapted to the coral system.
- **What management options can be applied to mitigate the effects of coral disease?** Sensitive, specific and robust coral disease diagnostics are an essential research priority, and once developed, these diagnostics can be applied to assess mitigation strategies including bacteriophage therapy or probiotic addition [62].

diagnosis and implementation of management strategies to either eliminate the disease from populations or greatly reduce its effects. The success in human and veterinary medicine is based on applying robust biomedical principles including rigorous anatomic descriptions of disease at the gross and cellular level and developing an understanding of the pathogenesis of disease and the interactions between agent, host and the environment. These principles as they apply to corals have been recently outlined [51].

The study of coral diseases is a young field of research. However, given the rate of loss of coral reefs, there is an urgent need for the research community to make significant and rapid progress. It is imperative that the small number of investigators within this field of wildlife disease investigations grow and recruit individuals skilled in a variety of disciplines, including ecology, veterinary medicine, microbiology, immunology, molecular genetics and mathematics. Happily, there is some light on the horizon. Organised research collaborations such as the coral disease working group of the Global Environment Facility Coral Reef Targeted Research Program have made significant steps in assessing the global range, prevalence and effects of coral disease while also standardising methods and nomenclature [7,52]. Systematic morphologic descriptions of coral diseases at the gross and cellular levels are required to enable comparative studies across large geographical regions and progress is being made on that front as well [53]. The Global Coral Reef Monitoring Network (GCRMN) has been working to develop standardized approaches for conducting coral disease research, including application of standardized nomenclature and diagnostic criteria. This has included the establishment of a detailed database aimed at collating information on the global distribution of coral diseases and contributing to the understanding of their prevalence. Importantly, records adhere to strict nomenclature and terminology, which allows general global comparisons to be made and represents an important step in collating relevant information to probe questions of causation (see http://www.unep-wcmc.org/).

In addition to applying standard biomedical tools, the use of new and emerging technologies applied to understanding causation of coral disease will be important for rapid advancement in this field. For example, molecular techniques including large metagenomic and transcriptomics projects alongside microarray studies allow characterization of functional changes of holobiont partners. These studies provide information on how such shifts affect whole animal fitness and their potential role in pathogenesis. A recent study by Vega Thurber and colleagues [54] demonstrated functional gene shifts in coral microbial partners including increase in abundance of virulence genes for coral undergoing temperature, nutrient and pH stresses. Sungawa and coworkers [55] used high-density 16S rRNA gene microarrays to document the shifts in the associated bacterial community structure in *Montastraea faveolata* colonies displaying phenotypic signs of white plague disease type II (WPD-II). These examples present comparative genomic approaches targeted at the bacterial component of the coral, although the techniques are equally valid when applied to other holobiont members. Such advanced methods will form the basis of an emerging understanding of how environmental stress and disease affect the genetics and physiology of a number of coral reef organisms [56]. There is immense potential for improving diagnostic detection of pathogens or host responses through the application of metabolomics – currently receiving great interest in human medical fields – with high resolution analytical techniques such as NMR and mass spectroscopy allowing quantitative detection of the chemical signatures of small molecules [57]. These techniques can be adapted in the future to coral pathologies once the causative agents are identified.

There is no doubt that this enhanced ability is beneficial, although it might complicate establishing disease causation [51], because there is still a need to distinguish between exposure to a potentially harmful agent and the actual disease caused by the agent. For example, molecular profiling of bacterial communities associated with corals that experienced a bleaching event correlated the relative increase in vibrios with initial visual signs of bleaching [30]. Such results might support the concept of bacteria-mediated bleaching [13], although this correlation does not demonstrate cause and effect. Therefore, linking presence of the putative agent to an appropriate pathological lesion remains important. Advanced microscopic techniques are another area of technological development that can support coral disease research. For example, fluorescent *in situ* hybridization (FISH) combined with species or genus specific rRNA oligonucleotide probes has already allowed for localization of bacteria within a specific coral lesion [29]. When the principles of classic biomedicine highlighted previously are applied alongside these rapidly advancing technologies, major advancements can be made in our understanding of coral pathology. The investigation of coral diseases, like that of other wildlife, human or veterinary pathologies, will remain an interdisciplinary endeavour using available traditional and developing technologies.

Concluding remarks

Understanding host–agent–environment interactions, pathology and factors that promote the virulence of the causative agent(s) are cornerstones of disease investigations. Many diseases in humans and animals are effectively managed because we understand this interaction and the causes of these pathologies. It has come to be expected that diseases within the human and some wildlife populations can be prevented or cured through drugs, vaccines or surgical intervention, although much of the improvement in human health is directly the result of improved sanitation, clean water and provision of safe food. Management of diseases within the marine environment in general and corals in particular, provide additional challenges because of the difficulties in controlling habitat and population dynamics and the potentially rapid rates of spread [58]. Management and prevention of coral diseases will be difficult unless we can generate the crucial data that allow us to understand the interaction between causative agents, corals and their environment. Until then, limiting human activities that have been demonstrated to have direct effects on coral health [43,59–61] is the priority in management practice and will only become more important as climate continues to challenge corals already living near their limits of thermal tolerance [40]. There are many questions to be answered (Box 3) and challenges to meet in the future to preserve coral reef ecosystems. Elucidating the causative agents of coral diseases is important, and only once this has been achieved can rapid diagnosis be applied for better management of these environments and response plans for specific outbreaks [62]. Other priority research areas include a full understanding of the coral holobiont with a coral microbiome project (similar to the ongoing human microbiome project) potentially contributing to elucidating the cellular mechanisms that regulate coral–microbe interactions in a healthy holobiont and the mechanisms disrupted during the onset of disease that perturb this normal homeostasis. The future research areas highlighted in Box 3 are broad, but all are a priority. These goals require recruitment of scientists into the field to achieve critical mass and rapid progress to mitigate and prevent coral decline caused by disease.

Coral disease research is a challenging and exciting field of animal disease investigations. It is a field in which scientists from many disciplines can make fundamental discoveries and rapidly advance scientific understanding while combining laboratory and field studies. These ecosystems are fundamental to regional economies and need to be preserved in the face of a changing world to ensure on-going sustainability of our marine resources. Understanding microbial diseases of corals therefore represents one important facet and a step towards preserving them for future generations.

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