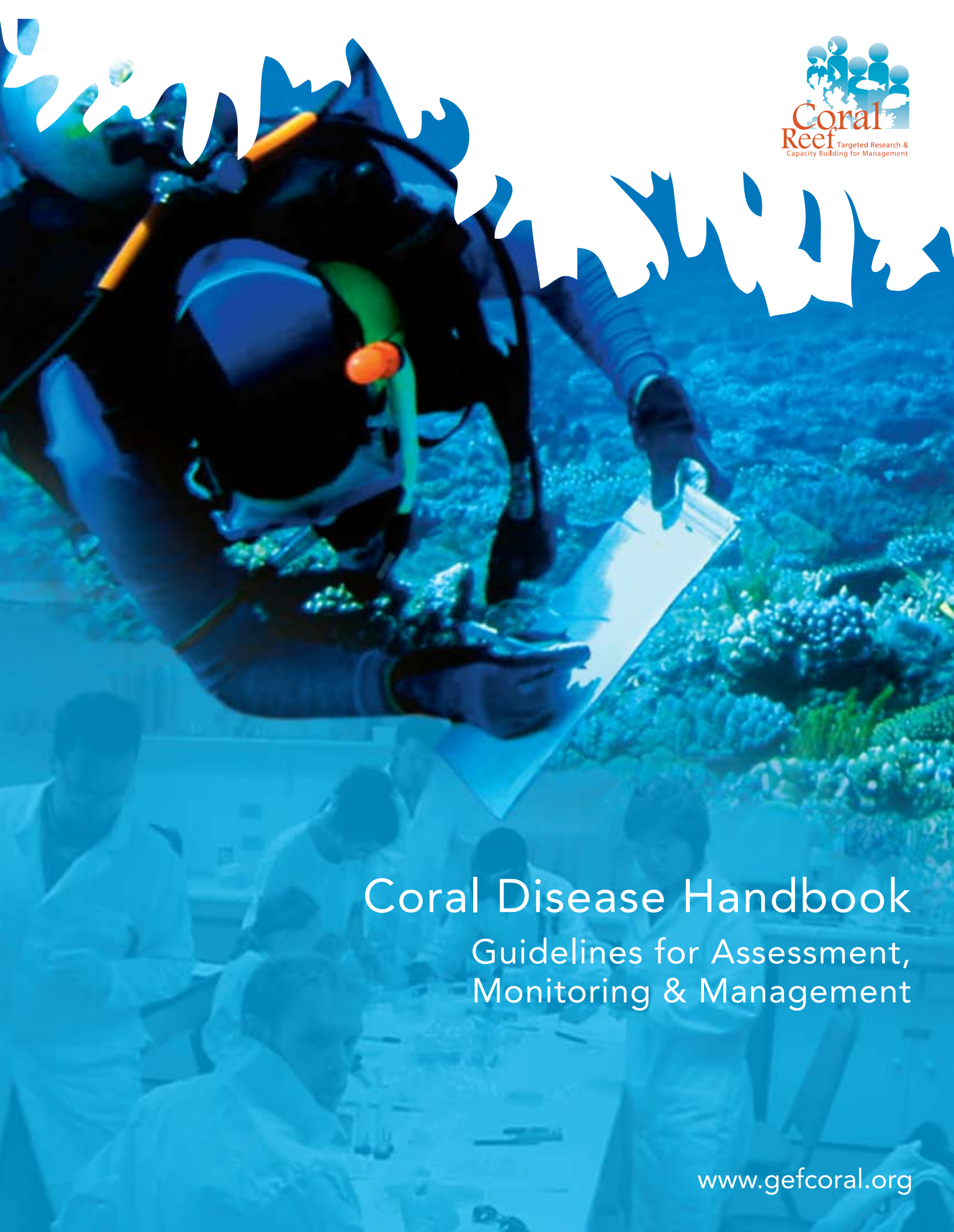

Coral Reef Targeted Research & Capacity Building for Management

Coral Disease Handbook

Guidelines for Assessment, Monitoring & Management

www.gefcoral.org

Editors: Laurie J. Raymundo, Courtney S. Couch and C. Drew Harvell

Contributing authors: Laurie J. Raymundo[1], Courtney S. Couch[2], Andrew W. Bruckner[3], C. Drew Harvell[4], Thierry M. Work[5], Ernesto Weil[6], Cheryl M. Woodley[7], Eric Jordan-Dahlgren[8], Bette L. Willis[9], Yui Sato[9], Greta S. Aeby[10].

Cover photos: (top) Lyle Vail, Lizard Island Research Station (A facility of the Australian Museum), (bottom) Ernesto Weil, University of Puerto Rico.

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[1]University of Guam, [2]Cornell University, [3]NOAA Coral Reef Conservation Program, [4]Cornell University, [5]USGS-National Wildlife Health Center, [6]University of Puerto Rico, [7]NOAA Center for Coastal Environmental Health and Biomolecular Research, [8]Universidad Nacional Autónoma de México, [9]James Cook University, [10]Hawaii Institute of Marine Biology.

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The University Of Queensland AUSTRALIA

The Coral Reef Targeted Research & Capacity Building for Management (CRTR) Program is a leading international coral reef research initiative that provides a coordinated approach to credible, factual and scientifically-proven knowledge for improved coral reef management.

The CRTR Program is a partnership between the Global Environment Facility, the World Bank, The University of Queensland (Australia), the United States National Oceanic and Atmospheric Administration (NOAA) and approximately 50 research institutes and other third-parties around the world.

Coral Reef Targeted Research and Capacity Building for Management Program, c/- Centre for Marine Studies, Gerhmann Building, The University of Queensland, St Lucia, Qld 4072, Australia
Tel: +61 7 3346 9942 Fax: +61 7 3346 9987 Email: info@gefcoral.org Internet: www.gefcoral.org

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Contents

Foreword

Our research careers began in Discovery Bay, Jamaica, in the mid 1970s, where we both studied the behavior of coral reef organisms, rather than the corals themselves. At that time, living coral covered 70 percent of the bottom, and no one worried about the long term persistence of the reefs, even though the reefs were clearly impacted by people via severe overfishing. Quite simply, we took the reefs for granted.

That sunny confidence turned out to be totally unfounded. In 1980, Hurricane Allen, a category five storm, struck and turned much of the reef into a rubble ground. However, reefs routinely get hit by hurricanes and typhoons, so they should have recovered. But in 1982 the sea urchin *Diadema antillarum* was decimated by an as yet unidentified pathogen, and losing this last remaining major grazer contributed to the overgrowth of corals by seaweeds throughout the region. By 1995, coral cover stood at less than 10 percent.

But the loss of grazers was not the only thing happening to these reefs. A more subtle and gradual but no less important killer was also taking its toll – the white band disease of the branching staghorn and elkhorn corals. These two species used to be so common that as students we were taught about the "*Acropora cervicornis* zone" and the "*Acropora palmata* zone". Now both species are listed as endangered under the Endangered Species Act, having lost over 90 percent of their numbers in the ensuing decades. Like the elms and chestnuts of US forests, they have largely vanished due to disease.

And they are not alone – white plague, yellow band, black band, and many others have since been documented as major reef killers, not only in the Caribbean but in the Pacific as well. For most of these diseases we still do not know the causative agent – nor the extent to which pollution and increased sea surface temperatures may be contributing to disease outbreaks or affecting the ability of corals to recover from infections. Yet progress is being made, and simply reliably recognizing and documenting these syndromes and their patterns of infection are important first steps in addressing this problem.

This handbook makes it much easier to do just that. Designed for managers, it outlines procedures for describing signs, measuring disease impacts, monitoring disease outbreaks, assessing causes, and managing reefs to minimize losses due to disease. As the authors note, information and expertise on coral disease are inadequate relative to the scale of the problem. This handbook helps managers not only to document and manage disease on the reefs they are responsible for, but also allows them to contribute to our scientific understanding of this grave threat.

Nancy Knowlton
Sant Chair in Marine Science
National Museum of Natural History
Smithsonian Institution

Marea Hatziolos
Environment Department
The World Bank

Chapter 1
The Objectives and Scope of This Manual

In this chapter you will find:

A general introduction to infectious diseases in corals – what they are, why they are a growing problem, and what is currently understood about them.

A look at the current global patterns and hotspots in regard to coral reef diseases.

A summary of the impact of ocean warming and poor water quality on coral reef diseases.

The Objectives and Scope of This Manual

L. Raymundo and C. D. Harvell

1.1 The state of coral reefs and the purpose of this manual

Coral reefs are the most diverse and among the most productive ecosystems on earth. Millions of people directly rely on the harvest derived from coral reefs as their major source of protein and income. In addition, the revenue coral reefs earn from tourism, recreation, education and research is of major importance to their local and national economies. And finally, current research in such areas as natural products chemistry suggest that coral reefs support an unknown number of organisms that may prove to be of major benefit in the treatment of critical human diseases. Yet, in spite of their obvious importance, reefs continue to be impacted by "the big four" human activities that threaten their sustainability: climate change, land- and marine-based pollution, habitat degradation and over-fishing.

Many of these impacts have obvious and immediate effects, such as smothering or fragmentation of coral to the point of total mortality. However, some effects, such as those from chemical pollutants, waste or excess nutrients, are more insidious, and their impacts may be more difficult to understand and quantify. One phenomenon which has recently gained the attention of coral reef scientists and managers is disease. Diseases affecting corals, particularly in the Caribbean, have increased in both frequency and severity within the last three decades and caused major community shifts on Caribbean reefs. Yet we are only beginning to understand enough about drivers of disease outbreaks to consider management actions.

While diseases affecting corals have increased since the 1970's, there are few individuals throughout the world trained to recognize diseases on coral reefs. In addition, there are many areas where there is absolutely no information regarding the status of coral health and disease.

Written for coral reef managers, this manual aims to fill the knowledge gap by bringing together what is currently known about coral diseases, how they are studied, and what options are available for managing them. We first present some general concepts about disease to put this manual and its scope in perspective. We then present the most current descriptions of known coral diseases, with information to assist in their field identification. Subsequent chapters are devoted to confirming field identifications, quantifying impacts of disease to coral communities, assessing disease on reefs, and setting up monitoring programs. We then provide information as to what is currently understood regarding disease outbreaks and how to track and study them. We end with guidelines on management practices and suggestions for where to obtain further information and direction.

Included in the appendices are categories of additional information which we hope will be useful. Underlined terms throughout the text indicate words listed in the glossary in **Appendix 1**.

1.2 What is disease?

Diseases are a natural aspect of populations, and are one mechanism by which population numbers are kept in check. For the purposes of this manual, we will use the term disease to mean "any impairment to health resulting in physiological dysfunction". Disease involves an interaction between a **host**, an **agent**, and the **environment**. The focus of this manual is *infectious biotic diseases*; those that are caused by a microbial agent, such as a bacterium, fungus, virus, or protist, that can be spread between host organisms and negatively impact the host's health. Other forms of disease that impact corals may be considered *abiotic diseases*; they do not involve a microbial agent but impair health, nonetheless. Examples may be those caused directly by environmental agents such as temperature stress, sedimentation, toxic chemicals, nutrient imbalance and UV radiation. In addition, *noninfectious biotic diseases* are not transmitted between organisms, though they may be caused by a microbial agent. For example, certain microbes secrete a toxin which damages the host animal or plant. A good example of this is botulism; toxins released by the bacterium *Clostridium botulinum* cause a non-infectious but deleterious disease in organisms that consume it.

1.3 Why study infectious diseases of corals?

Pathogenic microorganisms, having very short reproductive cycles, evolve more rapidly than multicellular organisms. They are also continually transported to new environments in the oceans by runoff, shipping vessels, aquaculture, and changing ocean currents. Therefore, we can expect that new diseases will continue to emerge. Recent examples of **emergent** infectious diseases on land that are threats to humans and wildlife include AIDS, bird flu, and SARS. Under specific conditions, disease levels may exceed a population's ability to cope, resulting in rapid and widespread mortality.

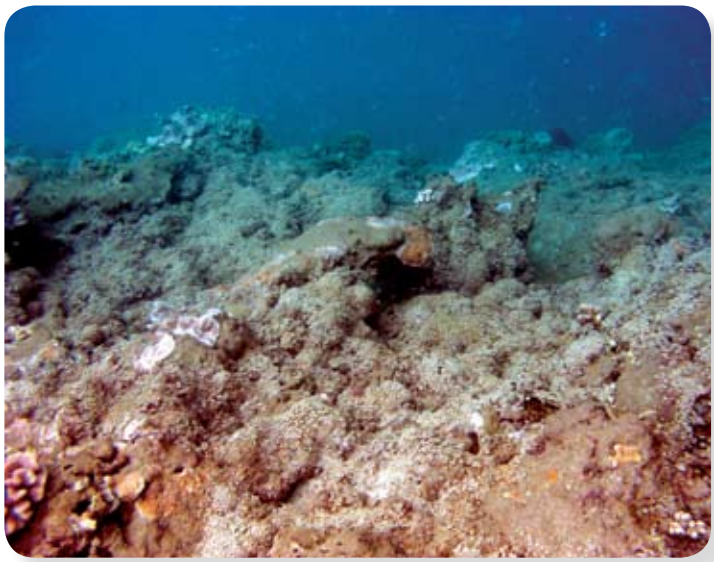


Figure 1.1 Reef in Hanalei Bay, Kauai, Hawaii, which has experienced extreme sediment stress, resulting in reduced coral coverage and the proliferation of the zooanthids. Photo: G.S. Aeby

A disease is considered an **outbreak** when the rate at which new hosts become infected increases. Technically, an outbreak is defined as R_0 >1. R_0 is the ratio of new infections to existing infections (see **Chapter 5** and **Appendix 1**).

Over the past three decades, coral reefs worldwide have experienced major changes in structure and function due to both anthropogenic and natural impacts (15-18). Virtually all of the most pervasive threats impacting coral reef ecosystems, including land-based and marine pollution, overfishing, global climate change, and ocean acidification, have been suggested as synergists or facilitators of infectious disease (**Figure 1.1**). Infectious disease in corals has increased in frequency and distribution since the early 1970's when a white band disease outbreak took a heavy toll on Caribbean acroporids. There has since been an exponential increase in numbers of reported diseases, host species and locations with disease observations. This rate of change is not normal, and has resulted in significant loss of coral cover.

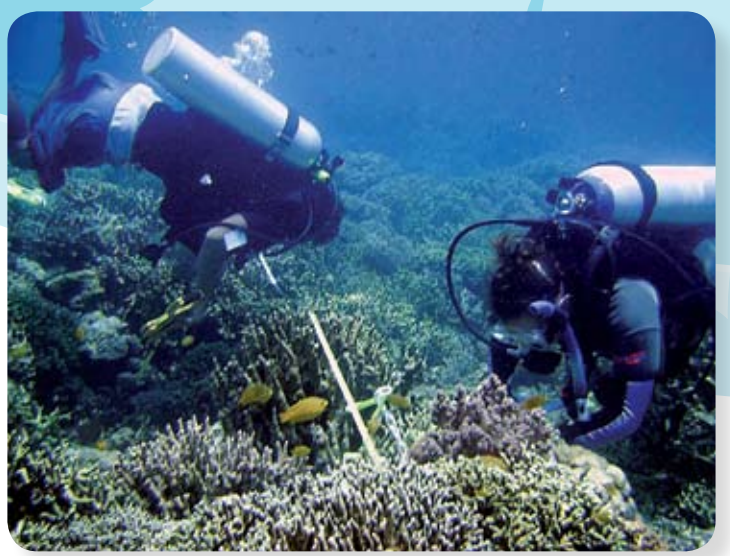


Figure 1.2 Students being trained in coral disease assessment methods in the Zaragosa Marine Protected Area, Central Philippines. Photo: L. Raymundo

Currently, the study of coral disease is in its infancy and those who devote their time and expertise to it are virtually "learning as they go along". However, through the experience of others who study and manage diseases in wildlife, farmed and cultured animals and plants, and even human populations, we can adapt methodologies and strategies to coral diseases that have been successful in other medical arenas.

This manual aims to address an urgent need: to update coral reef managers regarding our current understanding of the basic ecology of coral diseases. This will help improve monitoring efforts and aid in proper recognition of coral diseases and related issues of coral health (**Figure 1.2**). Although it is important to remember that detailed laboratory investigation remains essential for proper disease **diagnosis** and a complete understanding of the impacts to the coral host, we also hope that this manual will help increase the number of individuals able to provide information on the state of health of the world's reefs. By studying disease and establishing baselines prior to a crisis, we can arm ourselves with a better knowledge of appropriate management options for a given situation.

1.4 The emergence of coral disease

Damage to coral by abiotic and biotic factors acting alone or in synergy have led to a global reduction in coral cover (6,18,22-24). To date, the most infectious **syndromes** of coral for which a causative agent has been isolated involve bacteria (26). In addition to the loss of coral tissue, disease can cause significant changes in reproduction rates, growth rates, community structure, species diversity and abundance of reef-associated organisms (28,29). While an unprecedented increase in coral disease has been well-documented in the Caribbean over the last decade (11,25,30-32), and some argue that climate warming has driven part of the increase in damaging outbreaks (Causey, pers. comm.), much less is known about the status of disease throughout the Indo-Pacific (26). However, preliminary surveys in Australia (33), the Philippines (34), Palau (35), Northwestern Hawaiian Islands (36), American Samoa (37), the central Pacific (38), and East Africa (39,40), have revealed significant and damaging new diseases in all locations surveyed. Many of these are suspected or confirmed as infectious.

Figure 1.3 Coral bleaching within the Basdiot Marine Protected Area, Philippines, summer 2006. Photo: K. Rosell

What has prompted this emergence of coral disease? Current research suggests that humans may not only be introducing new pathogens into the oceans though aquaculture, runoff, human sewage, and ballast water, but may also be exacerbating existing **opportunistic infections** due to stressors such as poor water quality and climate warming (16,41). Climate warming is now established as an important factor in some current outbreaks (23,32,42). Some experts, such as Billy Causey (Superintendent, Florida Keys National Marine Sanctuary), argue that stressful warming events may have driven even more outbreaks than we have detected to date (Causey, pers. comm.). Because reef-building corals have a narrow range of thermal tolerance (between 18°C and 30°C), they are extremely susceptible to temperature stress. It is well known that corals "bleach" (lose their symbiotic zooxanthellae) at high temperatures (**Figure 1.3**). The coral bleaching observed worldwide following the 1998 El Niño was the most massive and devastating recorded up to that point (43), only to be exceeded by another bleaching event in Australia in 2002. The latter part of 2005 brought widespread bleaching to the Caribbean, caused by the largest warm thermal anomaly in 100 years (Eakin, pers. comm.). The Caribbean thermal anomaly of 2005 was immediately followed by outbreaks of white plague, yellow band disease (42) and white patch disease (32).

Our working hypothesis is that, in some cases, the death of coral during hot thermal anomalies is exacerbated by opportunistic infectious pathogens whose **virulence** is enhanced by increased temperatures. Changing environmental conditions could also influence disease by altering host-pathogen interactions. Increased temperatures could affect basic biological and physiological properties of corals, particularly their ability to fight infection, thus influencing the balance between potential pathogen and host (44). In addition, the pathogens themselves could become more virulent at higher temperatures (45). This is particularly challenging to study because of the complexity of the coral **holobiont**. The animal itself consists of the coral polyp, the unicellular algae (zooxanthellae) with which it co-exists in a **mutualistic** relationship, and a bacterial community existing within the surface mucous layer (SML), the coral tissue itself and its skeleton. This is very similar to the human **holobiont** that has its own unique and critical gastrointestinal mucosal microbiota which produces essential vitamins and amino acids not otherwise available to the human host. The coral SML contains a complex microbial community that responds to changes in the environment in ways that we are just now beginning to appreciate (46,47). The normal microbial flora within the mucus layer may protect the coral against pathogen invasion, and disturbances in this normal flora could lead to disease (48). The massive introduction of non-indigenous pathogens, which may occur with aquaculture and ballast water release, could also disturb the microbial community (16).

1.5 What is our current state of knowledge?

The current, and rather urgent, focus of research is the biology of microorganisms that can be pathogenic to corals. We are working diligently to develop new molecular and biomedical tools to identify specific agents and their origins, and determine the role of these agents in causing disease in corals. In **Figure 1.4**, we present five diseases with documented causal agents. The process by which causation is verified is explained in detail in **Chapter 3**. Undoubtedly as we learn more, we will continue to find that certain diseases may be caused by more than one microorganism, though whether this may be a matter of location, seasonality or other environmental parameters is unknown. For instance, the species comprising the microbial consortium associated with black band disease appears to vary with different geographic locations (49). Similarly, there is evidence that Caribbean yellow band disease (YBD) is caused by a consortium of bacteria (50). Because of inherent difficulties in the process, proving causation may be based on relatively few corals or disease events. For example, the demonstration of causation for both white plague type II and white patch disease are based on tests of relatively few corals, each from a single location or outbreak event. Our vision is that coral disease managers will eventually be equipped with molecular diagnostics to reliably verify the identity of a given infectious micro-organism. Thus the process of continuing to verify these agents is important (51).

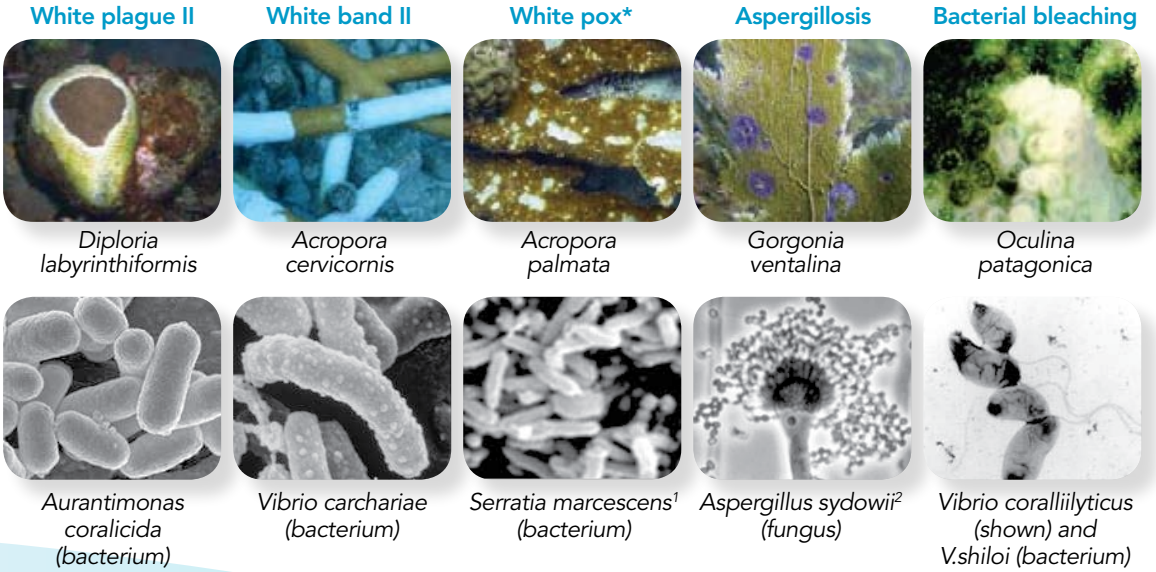


Figure 1.4 The five coral diseases for which Koch's postulates have been fulfilled, showing disease, host coral and microbial pathogen. The classic way to prove a microorganism causes disease is to satisfy Koch's postulates. A microorganism must be isolated from a diseased individual. That "isolate" is then used to infect a healthy individual. The same disease must develop, and the same organism must be isolated from the new infection. This classic method is a tough challenge in the face of unculturable marine microorganisms and polymicrobial syndromes, requiring molecular approaches.

*Originally named white pox, but field signs for this disease are now termed "white patch disease"; this name will be used in this book.
[1] source: http://commtechlab.msu.edu/sites/dlc-me/zoo/microbes/serratia.html
[2] source: http://www.cdc.gov/ncidod/dbmd/mdb/images/aspergillos.JPG
Harvell et al. (26). Photos by: A. Bruckner and E. Weil.

The last decade has been a time of intense research into causative agents of coral disease. Though we still lack evidence showing the origin of any coral disease, the role of specific pathogens in causing various diseases, their pathogenesis, and agent-host interactions, significant progress is being made in all of these areas. Some infectious agents that cause disease in marine animals, such as that of aspergillosis of **octocorals** (**Figure 1.5**) and toxoplasmosis in sea otters, are thought to originate on land.

Figure 1.5 Caribbean sea fan *Gorgonia ventalina* with multiple aspergillotic lesions. Photo: E.Weil

Others, such as viruses inadvertently introduced from shrimp or abalone farms to wild populations (McCallum, pers. comm.), originate in aquaculture farms (16). Tracking the origins of pathogenic agents might reveal sources that can be controlled before being introduced into the ocean. For example, *Serratia marcescens* is a ubiquitous bacterium introduced into coastal waters via sewage that may be the cause of white patch, a disease that affects *Acropora palmata* (52). There is a very real risk, therefore, that human activities may inadvertently introduce environmental stressors and potential pathogens to marine communities, and will continue to do so unless our understanding of such dynamics improves.

1.6 What are the global patterns and where are the hotspots?

The Caribbean has been referred to as a "hot spot" for disease because of a rapid emergence of new, extremely virulent diseases, increased frequency of **epizootic** events, and rapid spread of emerging diseases among new species and regions. At least 82 percent of coral species in the Caribbean are host to at least one disease (21).

In the Pacific, the threat of coral diseases has been regarded as minor, due to the large distances between reefs and island nations, fewer potential sources of pathogens, a paucity of epizootiological

studies and few recorded outbreaks. However, there were relatively few comprehensive detailed studies of coral disease in the Pacific prior to 2000, and most available information came from a handful of locations and researchers. As efforts increase to document coral diseases from more locations within the Pacific, the lists of species affected by disease, locations where diseases are reported, and **prevalence** of those diseases, are steadily increasing. It is now apparent that certain sites in the Pacific show a rather high prevalence of disease, and reports of outbreaks that kill a large number of colonies in a relatively short time suggest that the threat of disease impacts can no longer be considered minor.

1.7 What do we know about environmental drivers and stress?

An understanding of the influence that the environment plays in disease outbreaks could guide the development of useful management strategies (**Figure 1.6**). In this section, we summarize what is known about the relationship between particular environmental drivers and disease outbreaks. As with most aspects of the management of infectious disease in a marine setting, it is a work in progress and it is critical to keep in mind that all infectious syndromes are different and may respond in different ways to environmental change. However, identifying the factors that control the most important infectious syndromes is a key management strategy.

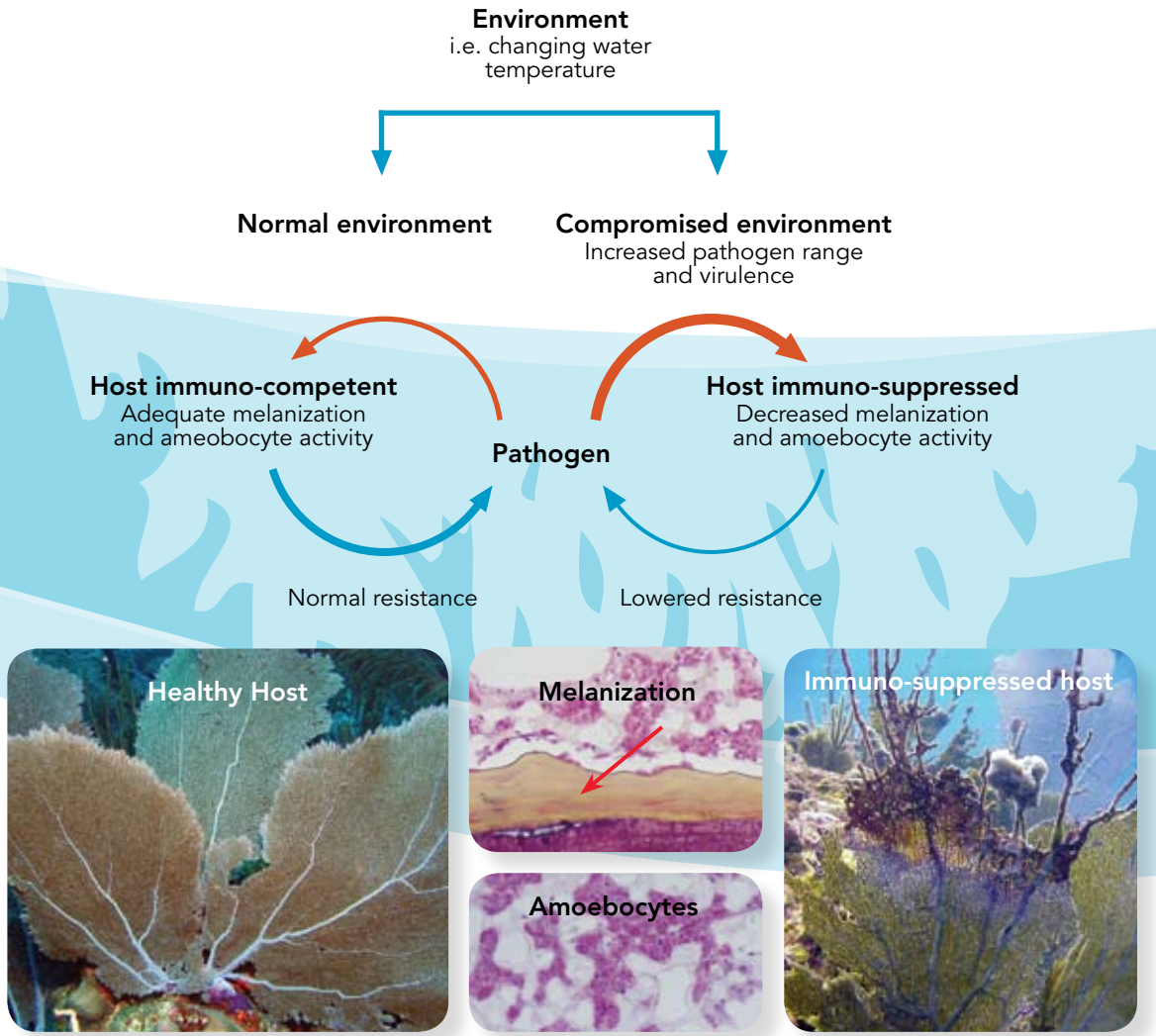


Figure 1.6 A schematic model showing the effect of an environmental impact – changing temperature – on a gorgonian coral infected by fungus. The healthy octocoral on the left is immuno-competent and is thus able to mount a normal immune response (melanization and amoebocyte activity). The diseased and dying octocoral on the right shows decreased melanization and suppressed amoebocyte activity, and is thus susceptible to attack by microorganisms. Modified from Mydlarz et al. (53). Photos by: C Couch and E. Weil.

Temperature

Outbreaks of some diseases are enhanced by ocean warming anomalies. An increase in disease following warming events may occur because corals are less able to fight disease while under temperature stress, or because pathogens are more virulent at higher temperatures. In three known cases where the pathogen can be cultured separately (*Aspergillus sydowii, Vibrio shiloi and Vibrio coralliilyticus*), pathogen growth and/or virulence increased with rising temperature, up to an optimal temperature (45,54-57).

Seasonal patterns in disease prevalence in the northeastern Caribbean provide further support for a link between warming ocean waters and disease outbreaks. Recurrent outbreaks of two virulent and damaging diseases, white plague and yellow band, have developed during seasons of highest water temperatures for the past four years on Puerto Rican reefs (Weil unpubl. data; Hernández-Delgado unpubl. data) and in the US Virgin Islands (42,58). Immediately following the peak of the 2005 bleaching event, the most devastating recorded in the North-eastern Caribbean, outbreaks of white plague, yellow band and white patch (32) were even more extensive in these areas and some outbreaks continued through 2007.

On the Great Barrier Reef, coral disease prevalence increased from winter to summer in all major families of coral (33). Prevalence increased fifteen-fold in acroporids, twelve-fold in faviids and doubled in pocilloporids in summer surveys. In addition, prevalence of three coral diseases increased significantly in summer surveys, with skeletal eroding band increasing more than two-fold, black band and other cyanobacterial infections more than three-fold, and white syndrome more than 50-fold.

Further work to document a link with temperature was carried out using disease prevalence surveys spanning 500 km of a latitudinal gradient along the Great Barrier Reef. In 1998, the Australian Institute of Marine Science's Long-Term Monitoring Program began to systematically monitor white syndrome (WS), which affects more than 15 coral species, including dominant plating acroporids. Divers conducted annual coral disease surveys on 47 reefs from 1998 to 2004 to quantify the number of cases of WS. Using a weekly four km data set of temperature values derived from the NOAA AVHRR Pathfinder (a radiation-detection imager that can determine sea surface temperature), a significant relationship was detected between the frequency of warm temperature anomalies and the **incidence** of white syndrome, indicating a relationship between temperature and disease. Interestingly, this relationship also depended on a high degree of coral cover, as would be expected for **transmission** of an infectious agent between hosts (23).

Links between outbreaks or increasing prevalence and warm temperature have thus been detected for black band disease, aspergillosis, yellow band disease, white patch disease and white syndrome. The list will likely grow as the data set expands. We still need to understand the mechanism operating in each syndrome: can we distinguish whether increased disease transmission during ocean warming is caused by compromised host **immunity** or the expansion of geographic range of microorganisms? Understanding these dynamics should aid in developing management strategies during periods of stressful temperatures.

Water Quality

As human populations continue to increase, nutrients, terrigenous silt, pollutants and even pathogens themselves can be released into nearshore benthic communities (59). While the link between anthropogenic stress and disease susceptibility is currently poorly understood, one hypothesis is that coral disease is facilitated by a decrease in water quality, particularly due to eutrophication and sedimentation. It is an urgent management priority to understand the link between water quality and infectious coral disease, because this is a local factor we can have some hope of managing.

Although corals are able to grow in high-nutrient water (60), recent evidence suggests a synergistic effect between elevated nutrients and disease. High nutrients (N, P) were associated with accelerated disease **signs** in both yellow band disease- and aspergillosis-infected corals in field manipulations (61), and in black band disease (62), although high nutrients alone were not associated with increased tissue loss in healthy corals. This is consistent with the findings of Kuntz et al. (63) who observed rapid tissue shedding in healthy corals exposed to elevated carbon sources, but little effect on corals of elevated N and P. Thus, corals seem to thrive under high nutrient conditions, but the combination of an active infection and elevated nutrients increases the disease **progression** rates of some syndromes. It is unclear whether this effect is due to an impact on host **resistance** or a positive effect on pathogen growth or virulence.

Figure 1.7 Tissue loss in a massive *Porites* in Palau caused by silt deposition. Photo: A. Croquer

Sedimentation offers yet another challenge to host disease resistance. The impacts of terrigenous sedimentation on nearshore communities are visible and well-documented; corals inhabiting silted reefs often possess large patches of dead, exposed skeleton bordered by apparently receding margins of healthy tissue (**Figure 1.7**). While coral tissue mortality was previously assumed to be the result of direct smothering, microbial agents may also contribute. Early work by Hodgson (64) identified silt-associated bacteria as a possible cause for **necrosis** in sediment-damaged corals, as antibiotic-treated water reduced the amount of tissue damage in experimentally-silted corals. More recently, opportunistic terrestrial pathogens (the soil fungus *Aspergillus sydowii* and the human enterobacterium *Serratia marcescens*) have been demonstrated as causal agents for two diseases currently impacting dominant corals in the Caribbean (52,65). Thus, terrigenous silt may not only cause physical stress for shallow, benthic organisms such as corals, but may also act as a pathogen **reservoir**.

This evidence suggests that anthropogenic stressors are linked with disease **severity** in complex ways. It is important to establish and quantify such linkages, as these factors may be possible to mitigate via improved reef management and land-use practices. The challenge lies in demonstrating these linkages in the complex system of diverse stressors acting upon the coral holobiont.

Box 1.1

Coral Reef Targeted Research: The Coral Disease Working Group

A Global Environment Facility/ World Bank initiative, the Coral Reef Targeted Research and Capacity Building for Management Program created six working groups to address the current alarming rate of reef decline by improving gaps in our knowledge of coral reef management (see www.gefcoral.org). As the Coral Disease Working Group for this project, the goals of our program are to fill critical information gaps about infectious coral reef disease, build capacity to study and monitor disease internationally, and help develop solutions for managing and conserving reef ecosystems. The cooperative research efforts are guided by our international team of microbiologists, ecologists and physiologists towards these ends. Working out of four Centers of Excellence, our research priorities include:

- assessing the global prevalence of coral disease;
- investigating the environmental drivers of disease;
- identifying the pathogens that cause disease; and
- understanding the coral's ability to resist disease.

We are also testing specific hypotheses about climate and anthropogenic changes that threaten coral reef sustainability. By building the capacity to manage these ecosystems, we hope to enhance reef resilience and recovery, worldwide.

Chapter 2

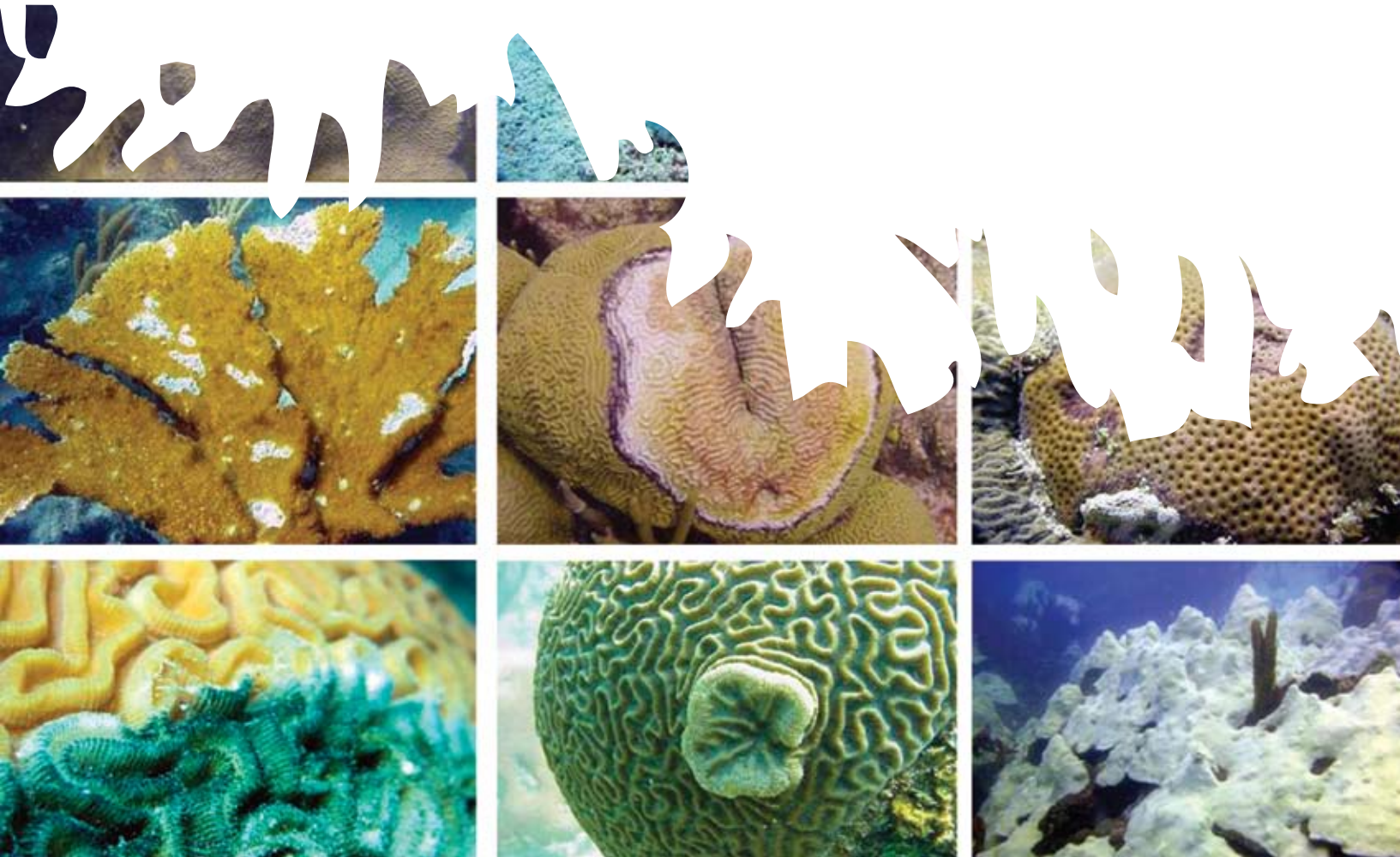
A Decision Tree for Describing Coral Lesions in the Field

In this chapter you will find:

A standardized procedure that will enable you to describe lesions in corals that encompasses the range of variation in colony morphology and geographic location.

Guidance for organizing and collecting data, particularly if you encounter a lesion that is unfamiliar or undescribed.

Descriptions and photos of commonly encountered lesions in the Western Atlantic, Indo-Pacific, Red Sea and East Africa.



A Decision Tree for Describing Coral Lesions in the Field

L. Raymundo, T. Work, A. Bruckner and B. Willis

2.1. Introduction

Disease is the absence of health and is usually manifested by the presence of a **lesion** (a morphologic abnormality). Three important points should be kept in mind when reading this chapter:

1. Diseases can have many causes; some of these are infectious (such as bacteria, parasites, or viruses) and others are not (such as genetically-based or toxicant-induced disorders).
2. The typical sign of a diseased coral is a lesion; a manifestation of disease that may not provide any clue regarding causation.
3. Some lesions in corals may have known causes that are not attributable to disease, though they result in the coral's health being compromised. For example, fish bites and crown-of-thorns starfish feeding scars should be characterized as predation; lesions associated with breakages may be caused by storms or anchor damage and should be characterized as disturbance; and lesions caused by aggressive interactions between corals or between corals and other sessile organisms should be characterized as competition. All can lead to tears and breaks in the tissue and partial mortality, and can **stress** the host coral. In suspected disease cases, it is often impossible to determine the cause of the lesion (and, therefore, the cause of the disease) without additional laboratory or experimental efforts (as discussed in **Chapter 3**).

Given the diversity of coral morphologies and the potential for environmental stressors to influence the progression of a disease, lesions may take on gross morphologies that differ between species or that vary temporally or spatially. The rapid growth of literature on coral diseases in the past few decades, in the absence of a standardized approach to describing lesions in corals, has resulted in a proliferation of disease names and confusion among researchers. The need for a standardized approach to describing lesions in corals is clear and urgent.

In this chapter we present a scheme that will allow you to describe lesions in corals in a manner that can be interpreted by others regardless of colony morphology or geographic location. This scheme also permits you to determine whether or not a lesion has a cause that can be readily determined with a high degree of confidence after a rapid assessment of the scene (i.e. predation, competition such as algal overgrowth, invertebrate galls). There are two compelling reasons for including lesions of known cause in your surveys:

1. Certain organisms that interact with corals may be **vectors** of disease or create potential entry wounds for infectious agents. Recording observations of such associations can lead to greater understanding of how a particular disease is spread, and thus is vitally important.
2. Documentation of such interactions indirectly provides information on ecosystem health. For example, a great number of lesions caused by smothering from silt may suggest that the reef is affected by land-based sedimentation. Reef managers could make use of this information to work with land managers and local legislators to improve land use practices because of a documented effect on coral health.

2.2 A decision tree for field-based assessments of diseases and compromised states of health

Presented here is a decision tree that outlines the steps needed to properly describe lesions in corals, applicable to reefs worldwide (**Figure 2.1**). It also provides a method for organizing information and offers a list of the types of data that are useful to collect if you encounter a lesion that is unfamiliar, or if a cause cannot be determined after an investigation of the scene. In such cases, it is critical to systematically describe what you see using the attached decision tree as a guide. Remember you will not be able to diagnose the cause of such lesions in the field without additional laboratory work. **Chapter 3** provides information on sample collection should you wish to submit specimens to a laboratory for analyses to prove causation.

After the decision tree, in sections **2.3** and **2.4**, you will find descriptions of commonly encountered lesions in the Western Atlantic and Indo-Pacific/East Africa/Red Sea respectively. These examples can be used to identify the diseases currently known for each region (additional information on these can be found in Appendix 6). Note that for each disease, a description of the lesion (compiled using the decision tree as a guide) is provided as an example.

For diseases that may be new or emerging, **Figure 2.1** provides a guide for describing the lesions observed. In all cases, **it is absolutely essential** to identify and record the host affected to genus (and species, if possible). Some diseases affect very few coral species while others appear to affect a wide range of hosts. Such information is invaluable in assessing impacts of a disease on a reef community, and is also important when evaluating the potential causes of disease in the laboratory.

Using the decision tree

1. Follow steps from 1 to 4 to identify and properly describe lesions in corals.
2. Use steps 4a ⟶ 4e to describe a lesion of unknown cause and determine whether it can be classified as any of the diseases described in **section 2.3** (Western Atlantic) or **2.4** (Indo-Pacific, East Africa and Red Sea).

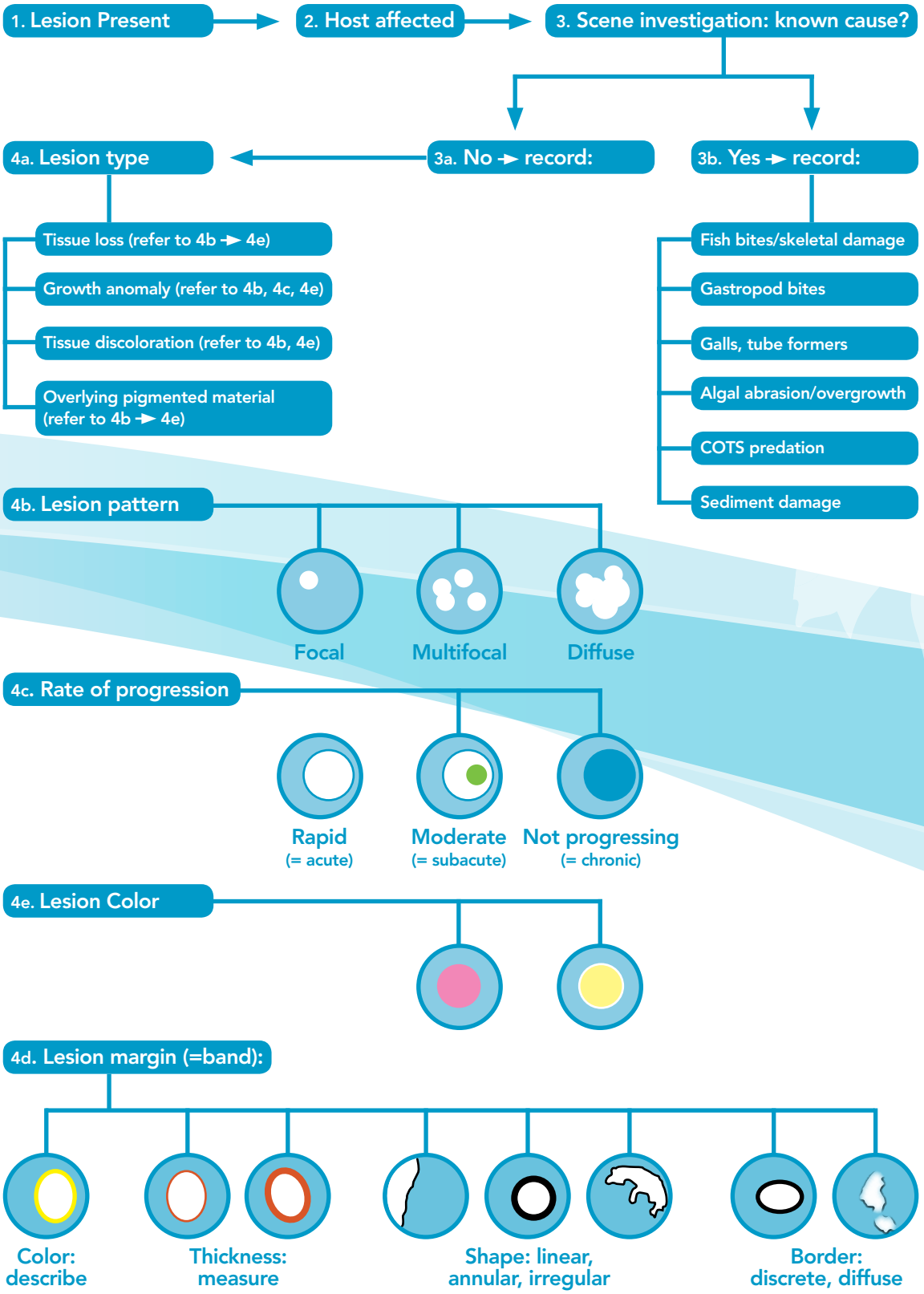


Figure 2.1 A globally-relevant decision tree used to identify known causes of lesions and describe lesions of unknown cause. All lesions denoted as white represent bare, exposed skeleton; green symbolizes secondary algal colonization of bare skeleton. Other colors represent examples of commonly-encountered lesions or legion margins characteristic of specific diseases.

2.3 Field assessments of Western Atlantic diseases and compromised health states

1. Tissue loss: known predation by fish and invertebrates resulting in compromised health

Fish bites

- Predominant corallivorous fishes including parrotfish, butterflyfish, filefish, pufferfish, triggerfish, and damselfish families.
- Corallivores may be in the surrounding area, but often are not observed feeding on coral.
- Most predators create distinctive scars characterized by removal of tissue and underlying skeleton. Butterflyfish delicately extract tissue from individual polyps without abrading the skeleton – these lesions are often only visible with a hand lens.

Below we describe the most common examples of fish predation encountered on western Atlantic reefs.

Parrotfish (focused biting)

- Diffuse patterns of tissue loss associated with scrapes or gouges (i.e. bite marks) by *Sparisoma viride* (stoplight parrotfish) that remove **corallites** and underlying skeleton.
- Lesions are large (2-50cm wide), and may be focal, multifocal or diffuse. Lesions often expand rapidly over one to five days, beginning at a focal point at the colony margin or within the colony surface and radiating out.
- *Sparisoma viride* graze predominantly on *Montastraea annularis, Montastraea faveolata, Colpophyllia natans* and *Porites astreoides*, and on 18 other species.
- In brain corals (C. *natans* and *Diploria strigosa*), fish remove tissue in a radiating band starting at one end of the colony. Look for predators in the area.

Spot biting

- Multifocal, paired lesions associated with removal of corallites, resulting from bite marks of parrotfish, pufferfish and other fishes.
- The size and shape of lesions may form a pattern consistent with the upper and lower jaw of the predator.
- Various species leave numerous bite marks on individual colonies.
- Scars include recent lesions lacking tissue and lesions in various stages of regeneration, as evidenced by pale tissue covering the injury.

Damselfish

- Multifocal well-circumscribed, circular, less than 1cm in diameter, acute to subacute (most species) or diffuse (brain corals) associated with tissue loss and removal of corallites by *Stegastes planifrons*.
- Lesions generally expand outwards, as older lesions are colonized by algae.

- In *Acropora*, coral growth over time may create chimney-like structures encircling the algae. In brain corals, bites follow ridges (previously misdiagnosed as "ridge mortality disease") – lesions progressively expand outwards, but tissue remains in grooves until overgrown by algae. In *M. annularis* species complex (shown) and *Siderastrea siderea*, fish bite at individual polyps in a mosaic pattern.
- Look for predators in the area.

Hermodice carunculata (fireworm or bristle worm)

- Diffuse acute tissue loss beginning from branch tips or colony projections, revealing intact underlying bare skeleton.
- The amphinomid polychaete *H. carunculata* feeds on less than 10 species of **scleractinians**, milleporids, anemones and gorgonians.
- Usually active only at night, but sometimes seen during the day.

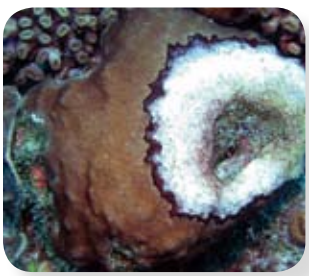
Gastropod predation

- *Coralliophila* is the only major genus that is predatory on Western Atlantic corals:
- Focal to multifocal, small, ovoid acute tissue loss. With heavy infestations, a scalloped pattern of shell scars may extend from the base or margin of the colony and radiate up and out.
- Two species commonly feed on corals. C. *abbreviata* (see arrow) feed on scleractinian and hydrozoan corals, while C. *caribaea* prefers gorgonians and zoanthids.
- Small individuals are relatively immobile and may cluster at colony margins.
- Snails may retreat to the base of the colony during the day.
- Look for predators on colony or at base.

2. Tissue loss: abiotic and biotic diseases

2a. Pigmented band diseases: the presence of a distinct narrow band of pigmented tissue

Black band disease



- Black or dark reddish-brown linear, diffuse or annular bands of acute to subacute tissue loss with a 1mm to 5cm wide margin, less than 1mm thick.
- Band is composed of black-red filamentous organisms peppered with white filaments, separating healthy tissue and white, bare skeleton.
- Band radiates outwards from the colony margin or a focal site of injury.

- In moderate (subacute) infections, denuded skeleton is colonized by filamentous algae and other epibionts.
- May be more than one disease front per colony which may merge over time. Affects 22 scleractinian corals, one hydrozoan coral and four octocorals.

Red band disease

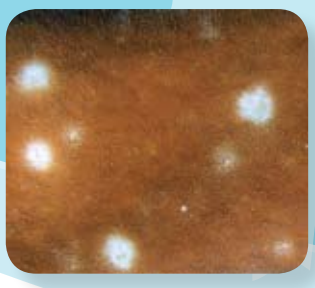
- Diffuse to circular band of red or dark reddish-brown filamentous organisms lacking white filaments, 1mm to 5cm wide.
- Rapid to moderate (acute to subacute) tissue loss reveals intact, bare to algae-covered skeleton.
- Band is linear to annular to irregular, radiating outwards from the colony margin or a focal site of injury.
- Common on octocorals, also affects Agaricids, *Meandrina* and *Mycetophyllia* and other less common scleractinians (see **Appendix 4**).

Caribbean ciliate infection

- Observed infecting coral in two distinct patterns: a diffuse black or grey band, several mm to 2cm thick, separating healthy tissue from bare skeleton or a diffuse scattered patch.
- Both bands and patches have a "salt-and-pepper" speckled appearance caused by the presence of ciliates.
- Patches may be associated with colonizing algae on bare skeleton

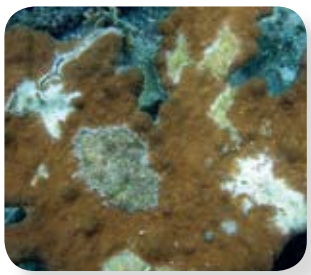
2b. Focal or multifocal tissue loss without distinct microbial band

Ulcerative white spots



- Multifocal well-circumscribed, distinct white discoloration or acute tissue loss revealing intact bare skeleton.
- Lesions are less than 1cm in diameter with discrete margin and may either contain bleached tissue or be devoid of tissue.
- Lesions may coalesce and become colonized by algae, or heal and disappear.

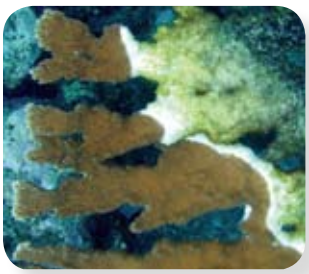
White patch disease



- Diffuse focal or multifocal lesions, 1-80cm in diameter with a sharply circumscribed leading edge of tissue loss.
- Lesions may radiate out over time and coalesce (see arrow), or (in *Acropora*) heal and resheet once mortality stops.
- Frequently, tissue remnants are visible adjacent to the leading edge.
- Corallites may be eroded, but underlying skeleton is intact.
- Formerly called white pox and patchy necrosis in *Acropora palmata*, but similar signs reported in other massive and plating corals.

2c. Annular or linear tissue loss without distinct pigmented band

White band disease



- Disease front characterized by linear, discrete band of acute tissue loss, 2-10cm wide, which may circumscribe the branch.
- Band separates healthy tissue from exposed skeleton colonized by epibionts.
- Disease progresses rapidly (mm-cm/day) from colony base or branch bifurcation.
- Tissue adjacent to exposed skeleton may be bleached; snails and fireworm predators may colonize the disease front.
- Only observed in *Acropora*.

White plague

- Lesions are focal or multifocal-to-coalescing, with a linear or annular margin, depending on colony morphology.
- A discrete band of bare skeleton separates live tissue from algal-colonized skeleton.
- Tissue adjacent to exposed skeleton may be bleached.
- Linear tissue loss begins at the base or margin of a colony, or emanates from an algal/sediment interface within the colony, and advances 1mm to > 10cm/day.
- Closely resembles white band disease, but affects more than 40 spp. of non-acroporid massive and plating corals.

2d. Tissue loss without distinct pigmented band

Caribbean white syndromes

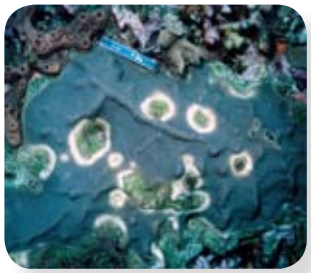
- Diffuse patterns of tissue loss with no distinctive pigmented mat or band at the interface, i.e. tissue loss that is not characteristic of either white band or white plague.
- In acroporids, this can include diseases that start within the colony and not at the base, and spread in irregular patterns.

3. Discoloration

Dark spots disease

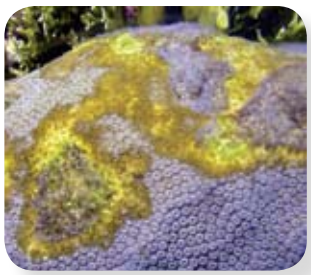
- Focal to multifocal lesions with annular to irregular margins, purple to brown in color and 1cm to more than 45cm in diameter.
- Dark spots may expand over time, coalesce, and form diffuse to annular bands adjacent to or surrounding exposed skeleton.
- Affected tissue may be associated with a depression of the coral surface and may seasonally disappear.
- Underlying skeleton may retain dark pigmentation when tissue is gone.
- Primarily affects *Stephanocoenia*, *Montastraea* and *Siderastrea*.

Yellow band disease



- Focal, multifocal, diffuse lesions with annular to linear margins of pale yellow, bordered by healthy tissue.
- Lesions progress mm to cm per month.
- The leading edge of the band remains pale yellow or lemon colored, while tissue previously affected gradually darkens prior to full tissue loss; acute tissue loss is rare.
- Primarily affects *Montastraea*.

Pigmentation response



- Multifocal or diffuse areas of white, purple, yellow, brownish or blue colored tissue discoloration.
- Tissue may appear unhealthy, swollen, and/or peeling away at the edges.
- Pigmentation may form lines, bumps, spots, patches, bands or irregular shapes.
- Considered a response of the coral host to a variety of stressors (i.e. unidentified pathogens, competition, predation, boring fauna, abrasion, etc.), suggesting that organism health is compromised.
- Common on corals such as *Porites*, *Siderastrea*, and *Montastraea* and octocorals such as *Gorgonia*, *Pseudoplexaura*, *Plexaura*, *Briareum*, and *Erythropodium*.

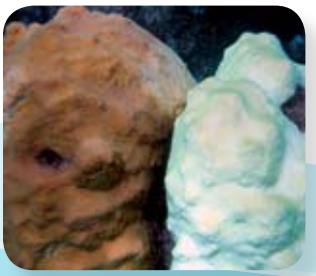
Aspergillosis

- Diffuse lesion(s) of various sizes and shapes distributed throughout the sea fan blade and branch network, resulting in loss of tissue and/or skeleton.
- Tissue surrounding the lesion often becomes dark purple (pigmentation response). Affected colonies may also produce purple nodules or galls near the lesion, which can encapsulate fungus, algae or other epibionts in an attempt to confine the infection.
- Lesions recently produced by predation (flamingo tongue, fireworms) usually do not show purple coloration but instead the dark brown skeletal matrix, devoid of tissue, is clearly seen.
- Some of these lesions along the branches eventually produce purpled edges.
- Lesions from continuous contact with other octocorals, corals, hydrocorals and/or the substrate usually show the pigmentation response at the point of contact.
- Only affects octocorals, most commonly *Gorgonia, Pseudopterogorgia, Plexaura, Plexaurella*.

Ulcerative white spots

- Described above under Tissue Loss.
- Also involves loss of pigmentation, as lesions may contain bleached tissue at certain stages, so it is cross-referenced here.

Bleaching



- Focal, multifocal-to-coalescing, or diffuse areas of tissue discoloration.
- Loss or reduction in the number of endosymbiotic algae (zooxanthellae) from coral tissue.
- Tissue is present, but with reduced or absent pigmentation.
- Bleaching can affect the entire colony, upper surfaces, the base, or discrete patches.
- Bleached tissue may be associated with irregular patterns of tissue loss.

4. Growth anomalies

Galls

- Focal to multifocal skeletal deformation with presence of organism (crab, barnacle, etc.).
- Deformations caused by skeletal deposition around the resident invertebrate result in uncharacteristic patterns. Resulting lesions may be focal or multifocal, circular to irregularly shaped mass of thickened coenosteum (see arrow), elevating polyps 2-4mm above the surface of the colony or fan.
- Also reported as tumor-like growth, tumor, algal tumor, algal gall, gorgonin pearl, and nodules on gorgonians.

Growth anomalies of unknown cause

- Focal or multifocal, annular to diffuse lesion consisting of abnormally arranged skeletal elements (corallites, ridges, valleys), which are visibly larger or smaller than those of adjacent healthy tissue.
- They may protrude above the colony surface, and may or may not be covered by intact tissue.
- Pigmentation may be normal, lighter (suggesting loss of zooxanthellae), or completely absent (suggesting an absence of zooxanthellae).
- In some types, corallites may be completely absent, and the growth anomaly resembles a white plaque over the colony surface. In other types, corallites may be highly disorganized and tissue may die in irregular patches and bare skeleton may be colonized by epibionts.
- Also includes conditions referred to as gigantism, accelerated growth, tumors, and chaotic polyp development.

2.4 Field assessments of Indo-Pacific, East African and Red Sea diseases and compromised health states

1. Tissue loss: known predation or stress resulting in compromised health

Fish bites

- Look for predators in survey area (though they may actively feed at night) and distinctive scars on coral skeleton.
- These examples are not exclusive and other fish predators may leave different scars.
- Look for gouging, scraping, or other regular patterns of tissue loss, often clustered on colony surface.

Below we describe common examples of fish predation encountered in the Indo-Pacific, East Africa and Red Sea regions.

Parrotfish

- Diffuse patterns of tissue loss associated with scrapes or gouges (i.e. bite marks or scars) that expose bare skeleton.
- Recent lesions are white and typically have discrete borders.
- Older lesions may be healing or partially or wholly colonized by algae, the latter indicating that tissue loss is not progressing.
- Scars may be focused along exposed ridges of coral.
- Parrotfish are usually in the vicinity and feed by day.

Pufferfish

- Multifocal, linear to oblong paired areas of distinct tissue loss with mild erosion of bare skeleton (see arrows).
- Pufferfish may be in vicinity, but may not be observed feeding.
- Less damaging to skeleton than parrotfish bites, and may also be concentrated along exposed colony ridges.

Damselfish

- Diffuse patterns of tissue loss producing lesions that may be linear, annular or irregular in shape.
- Lesions are colonized by algae that are farmed by damselfish visible in the area.
- Most frequently observed in branching *Acropora* thickets.

Acanthaster planci (Crown-of-Thorns starfish; COTS)

- Diffuse morphous area of tissue loss revealing intact, bare skeleton.
- Lesion margin may be scalloped (see arrow) on plating, massive or tabular colonies.
- Lesion border is generally discrete and may have visible strings of tissue and mucus.
- Feeding usually occurs from colony edge (plating massive, tabular forms) or base (branching forms), exposing large areas of white skeleton consistent with rapid tissue loss.
- COTS are in vicinity either feeding or under colony by day.

Tube formers

- Focal to multifocal, circular to amorphous areas of tissue loss with erosion of skeleton and annular thin band of white or pink tissue accompanied by presence of boring polychaetes (tube worms – see arrow), gastropods (vermetids), or barnacles.
- Feeding structures and gills protrude from the coral surface. Common on massive *Porites*.
- Also observed in the western Atlantic.

Gastropod predation

The following two genera are major predators of Indo-Pacific corals (limpets and other molluscs are also known corallivores):

Drupella

- Diffuse areas of tissue loss extending from branch bases or colony edges, revealing bare, intact skeleton (see arrow).
- Lesion has a discrete border, and strings of mucous and tissue may be visible.
- Rate of tissue loss typically slower than for *A. planci* predation, though during outbreaks, numbers per colony may be in the hundreds.
- *Drupella* in vicinity hiding at colony base by day, often clustered, or feeding by night. Empty shells also indicate presence.

Coralliophilia

- Focal to multifocal areas of tissue loss revealing bare eroded skeleton and occasional raised thin pink annular band (pigmented coral tissue encircling lesion).
- Shells are relatively immobile and firmly attached to colony surface; may be heavily fouled and more visible on massive corals.
- May be clustered in colony crevices and show strong preference for massive and branching *Porites*.
- Old feeding scars may be present (see arrow).

Sediment damage

- Diffuse amorphous area of tissue loss revealing skeleton covered by sediment.
- Water is typically highly turbid and sediment visible on benthic surfaces. When it accumulates on live coral, it leaves dead, fouled skeleton underneath.
- Also observed in the western Atlantic.

Algal overgrowth

- Colonization and overgrowth of living coral tissue by algae (various species).
- With heavy overgrowth, underlying coral tissue usually dies, leaving bare skeleton.
- Abrasion may cause a pigmentation response (see below under Discoloration), but this is not always present.
- Also observed in the western Atlantic.

2. Tissue loss: abiotic and biotic diseases

This refers to lesions that do not have any of the discrete patterns of tissue loss or skeletal damage consistent with predation or compromised health states described above.

2a. Pigmented band diseases: presence of a distinct narrow band of pigmented material

Black band disease

- Black or dark reddish-brown linear or diffuse annular bands at the interface between live coral tissue and exposed skeleton (see arrow).
- Band comprises black-red filamentous organisms (cyanobacteria) peppered with white filaments which can only be seen microscopically.
- Band radiates outwards from the colony margin or a site of injury on massive, plating or foliose corals, or circumscribes branches on branching corals.
- In moderately progressing infections, denuded skeleton is colonized by filamentous algae and other epibionts.
- May be more than one disease band per colony which may merge over time.
- Affects at least 40 species of corals, particularly *Acropora* species.
- Also observed in the western Atlantic.

Skeletal eroding band

- Black or dark green "salt-and-pepper", speckled, diffuse band.
- May form either a discrete, dark band several mm to cm wide at interface between healthy tissue and recently exposed skeleton (1o infection; photo) or a diffuse, scattered patch on exposed skeleton (2o infection following predation or other tissue loss).
- Speckled appearance caused by boring ciliates which erode skeleton.
- Common in *Acropora* and *Pocillopora*.

Brown band

- Brown, linear or annular band at the interface between live tissue and exposed skeleton, though a thin white band between brown band and healthy tissue is sometimes also present.
- Lesion border is typically discrete.
- Tissue loss may be rapid and begins from branch base but may spread to adjacent branches at contact points.
- Band consists of mobile ciliates, which may contain zooxanthellae from consumed tissue (visible under microscope; gives band its brown color).
- Observed most commonly on branching *Acropora*.

2b. Tissue loss without distinct band

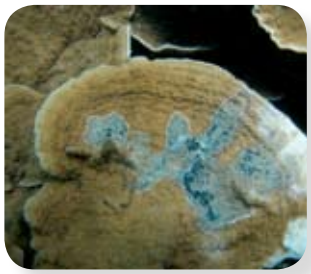
Ulcerative white spots

- Multifocal patterns of tissue loss exposing intact, bare white skeleton.
- Lesions are small (<1cm diameter), regularly ovoid, with discrete margins and may either contain bleached tissue or be devoid of tissue.
- Heavy infections may result in lesion coalescence (see arrow) followed by algal colonization.
- Most common on *Porites*; also on *Montipora*, faviids, and the octocoral *Heliopora*.
- Also present in western Atlantic.

White syndromes

- Diffuse areas of tissue loss exposing bare, intact skeleton.
- No band apparent between healthy tissue and bare skeleton; lesion border may be discrete or diffuse, but not pigmented.
- Rate of tissue loss moderate to rapid.
- Lesions behind active disease fronts are white, grading to brown distally as skeleton becomes fouled. Can resemble bleaching, but close inspection reveals absence of tissue.
- Host range wide, affecting at least 15 genera.

Atramentous necrosis



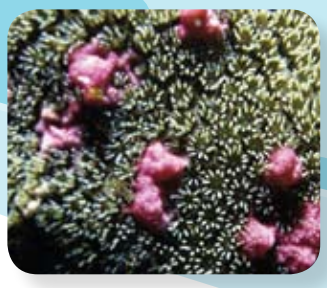
- Multifocal to irregular pattern of tissue loss exposing bare, white skeleton subsequently colonized by a distinctive grayish-black, fouling community.
- Lesions typically start as small bleached spots followed by tissue loss and coalescence of adjacent lesions.
- Bare skeleton may be covered by a thin white film, under which a black sulfurous deposit may accumulate, giving the lesion a grayish appearance.
- **Chronic infections** result in colonization by epibionts which obscure typical signs of disease.
- *Montipora* are most susceptible, but it has also been observed on *Acropora*, *Echinopora*, *Turbinaria* and *Merulina*.

3. Tissue discoloration

Pigmentation response

- Multifocal or diffuse areas of pink, purple or blue brightly colored tissue discoloration.
- Tissue on corallite walls may appear swollen or thickened. Pigmentation may form lines, bumps, spots, patches or irregular shapes.
- Considered a response of the coral host to a variety of stressors (i.e. competition, boring fauna, algal abrasion – see arrow), suggesting that coral health is compromised.
- Common on *Porites*, which displays bright pink or purple pigmentation.

Trematodiasis



- Multifocal, distinct pink to white, small (1-2mm) areas of tissue swelling.
- Swelling is a response to presence of an encysted parasitic trematode (flatworm) visible under microscope if tissue is sampled.
- Only observed on massive *Porites*.

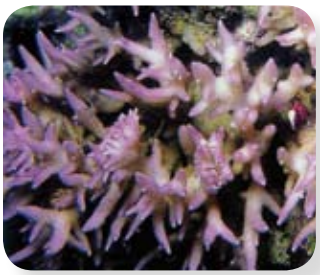
Unusual bleaching patterns

- Diffuse focal, or multifocal-to-coalescing amorphous areas of white tissue with a discrete margin.
- Loss or reduction in the number of endosymbiotic algae (zooxanthellae) from coral tissue. Note that tissue is present, but with reduced or absent pigmentation.

- Distinguished from thermal bleaching which typically affects upper or entire surfaces of corals. Unusual bleaching patterns include white stripes or patches often with discrete borders.
- The degree of bleaching can vary from pale to white, and indicates compromised health.

4. Growth anomalies

Galls



- Focal to multifocal skeletal deformation associated with the presence of an organism (i.e. crab, barnacle, etc.).
- Deformations caused by skeletal deposition around the resident invertebrate in uncharacteristic patterns. Resulting lesions may be focal or multifocal, circular to irregularly shaped masses of thickened coenosteum (see arrow), elevating polyps several mm above the surface of the colony.
- Also present in the western Atlantic.

Growth anomalies of unknown cause

- Focal or multifocal, circular to diffusely shaped lesions consisting of abnormally arranged skeletal elements (corallites, ridges, valleys), which are larger or smaller than those of adjacent healthy tissue.
- They may protrude above the colony surface, and may or may not be covered by intact normal-appearing tissue.
- Pigmentation may be normal, lighter (suggesting loss of zooxanthellae), or completely absent (suggesting absence of zooxanthellae). In some corallites it may be completely absent, and the growth anomaly resembles a white plaque over the colony surface. In other types, corallites may be highly disorganized and tissue may die in irregular patches and bare skeleton may be colonized by epibionts.
- Also includes conditions referred to as: gigantism, accelerated growth, tumor, neoplasia, hyperplasia, chaotic polyp development.
- Also present in the western Atlantic.

Chapter 3

Confirming Field Assessments and Measuring Disease Impacts

In this chapter you will find:

Key questions to ask when disease is detected: geographic extent, host range, seasonality, and case fatality rate.

Basic methods for describing lesions in corals and confirming field assessmnts.

A summary of current practices used to determine the causes of unknown lesions in corals.

Information on how to collect, handle and preserve specimens for histology, microbiology and molecular assays and general considerations such as safety, permits and labelling.

Confirming Field Assessments and Measuring Disease Impacts

T. Work, C. Woodley and L. Raymundo

3.1 Monitoring changes over time

Three questions are of paramount importance to resource managers when disease is detected in an animal population:

1. How widespread is the disease (geographic extent)?
2. Is the disease spreading and if so, how fast (geographic spread)?
3. Is the disease killing animals (case fatality rate)?

To answer these questions, the state of disease in the ecosystem must be monitored over time. Deciding how frequently to monitor is dictated primarily by the behavior of the disease in the field. For example, diseases that appear to be spreading rapidly will need more frequent monitoring than those that are spreading slowly or appear static. It is important during this phase of monitoring to liaise with appropriate experts who can help determine the cause of disease (refer to **Appendix 2**). This will, at a minimum, necessitate collection of samples for laboratory investigations (see **Section 3.4** of this chapter). **Chapter 4** provides guidance in developing a monitoring program to help you address impacts of disease on coral communities.

1. Geographic extent

To determine the regional extent of a disease requires that you know the location of populations at risk of disease; those most likely to be affected by the disease. Criteria that should be considered when deciding which areas to survey for extent of disease include, but are not limited to, percent coral cover, species richness, proximity to the original site of disease detection, susceptibility of the population, and accessibility of the site. Depending on how the disease is behaving, some criteria may take priority over others. If the disease affects multiple genera of corals, those reefs with the highest coral cover would be prioritized for supplementary surveys (**Figure 3.1.A**). If the disease only affects a single genus, reefs with high cover of that particular genus would be prioritized for supplementary surveys (**Figure 3.1.B**). If field evidence suggests the disease may be infectious (i.e. a lesion is observed spreading over time from a diseased coral to adjacent colonies), priority would be given to surveying adjacent reefs or those down-current (**Figure 3.1.C**). The extent of disease can be tracked spatially using commonly available geographic information systems software (GIS) tools.

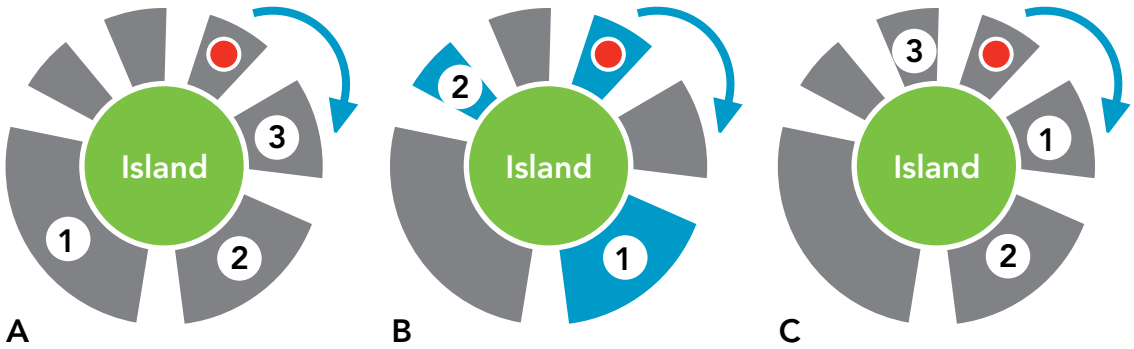


Figure 3.1 Hypothetical island with fringing reefs (grey areas), prevailing ocean currents (blue arrow), and reef with disease (red dot). Numbers indicate order of priority for survey sites. **A)** If disease appears to affect all corals, then surveys to assess extent are targeted for those reefs with highest coral cover. **B)** If disease affects only one particular genus, then surveys to assess extent are targeted to those reefs that have highest cover of that genus (blue areas). **C)** If disease appears infectious, surveys are targeted to adjacent reefs down and up stream of the diseased reef.

When assessing the geographic extent of disease, managers should address the following questions (listed under the headings "Hosts", "Place" and "Time"). The answers may provide clues to laboratory diagnosticians on potential causes of disease.

Hosts

- *Does disease primarily affect a particular group or genus of corals*?

  Some diseases, particularly those caused by infectious agents, are very host-specific. On the other hand, if multiple hosts are affected, this may indicate a non-specific cause of disease (i.e. elevated temperature, poisoning, etc.).

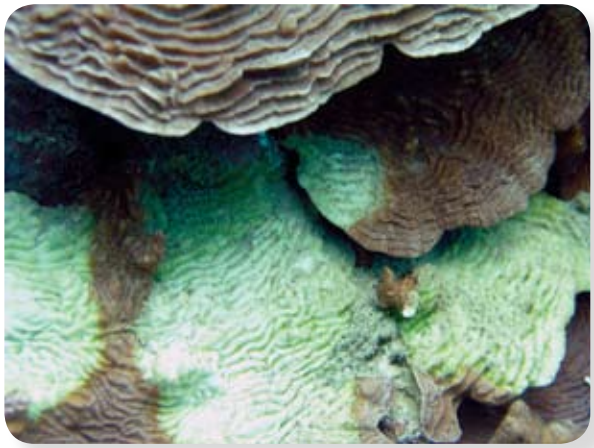


Figure 3.2 White syndrome spreading to adjacent colonies of *Pachyseris* in Palau. Photo: L. Raymundo

- *Does disease primarily affect a particular size class of coral?*
  Certain diseases affect older colonies more than younger ones and vice versa.
- *Does disease appear to be spreading between adjacent colonies?*

  Evidence of this is strongly suggestive of a communicable agent (Figure 3.2). Note that this needs to be confirmed through additional laboratory investigations.

Place

- *Do corals affected by the disease have a particular spatial distribution?*

  For example, perhaps colonies that are shaded are more prone to disease. Perhaps disease appears to affect colonies predominantly on the reef flat but those on the reef crest or slope are unaffected. Perhaps there is a depth or water circulation gradient associated with disease occurrence.

- *Have there been recent changes in the environment?*

  Answering this could partly explain why disease suddenly occurs at a particular location. For example, have there been recent changes in land use patterns adjacent to the reef (chemical spills, construction, excessive terrestrial runoff) or unusual environmental events (hurricanes, temperature anomalies, fresh water influxes)? It is important to collaborate with local agencies and other managers who are responsible for monitoring environmental characteristics such as rainfall, water temperature, turbidity and salinity (see Chapter 4 for details). Keeping track of such data, along with changes in disease prevalence or incidence, can often reveal patterns which may be exacerbating or inhibiting the impact of the disease.

Time

- *Does disease occur more frequently during certain times of the year?*

  Some diseases have a seasonal component. Determining whether a temporal component exists could provide clues to potential causes of disease, and will help to predict future impacts.

2. Geographic spread

Visual surveys of geographic extent over time can give an indication of how fast a disease is spreading. However, it is usually more desirable to quantify the spread of disease in a population, particularly when one is comparing multiple regions or sites. One practical way to do this is to measure the incidence of disease. Unlike prevalence, which is a static measure (of disease), incidence measures the number of new cases of disease over a defined time period and thus can be a useful indicator of whether or not disease is spreading. Increasing incidence suggests a spreading disease. By its very nature, incidence (of disease) can never be greater than prevalence (see **Box 3.1**). See **Chapter 4** for calculations of disease prevalence, incidence and other parameters.

3. Case fatality rate

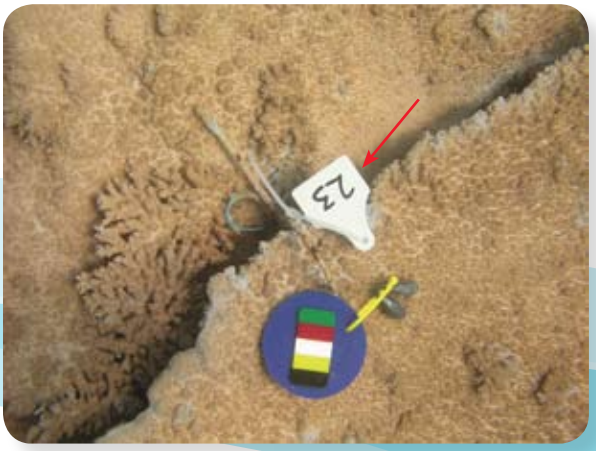


Figure 3.3 Cattle ear tag (see arrow) used to mark and code a coral colony. Photo: T. Work

This is measured as the percent of colonies having a disease that actually die from that disease. Managers should be more concerned with diseases that have a high case fatality rate (see **Box 3.1**). To get a measure of case fatality rate, it is necessary to mark colonies affected with disease and to measure the number of colonies with disease that die after a set time period. Various methods exist to mark coral colonies (i.e. masonry nails, flagging tape, plastic or stainless steel tags), but we have found that commercially available cattle ear tags affixed to colonies with cable ties will last for at least one year, even in highly turbulent conditions (**Figure 3.3**).

One last note on corals

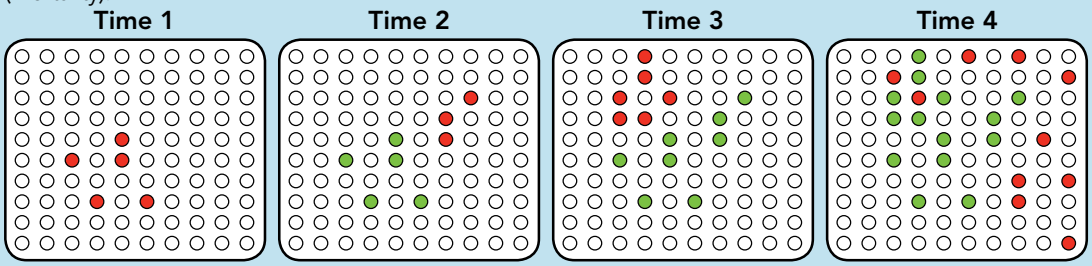
Corals are colonial animals that fragment as a means of asexual reproduction. They can also partially die and later grow new tissue over this dead skeleton; both processes can slow growth of the colony as a whole and reduce colony size. The age of a colony may, therefore, be difficult to estimate from colony size. While it can be assumed that large colonies of a given species are old, small colonies are not necessarily younger; they could have experienced fragmentation or partial mortality or other factors which have resulted in slow growth rate. While measuring coral colony sizes is reasonably straightforward, it is important to remember that estimating age from size should not be attempted.

Another factor which can be challenging when assessing disease in a coral community is colony boundaries. With extensive monospecific stands, it may be difficult to determine where one colony ends and another begins, particularly when colonies are in physical contact. When faced with this situation, look for subtle changes in coloration; often different colonies will appear as slightly different shades. Also, look for the borders where the colonies abut each other; if a stand or thicket is composed of different colonies (rather than a single one), then these colony margins will not fuse, but will remain separate, and may be differentially pigmented or show signs of competition.

Box 3.1

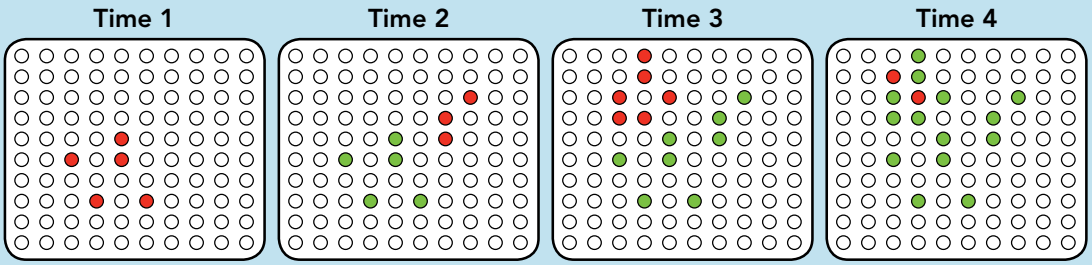
Examples of prevalence and incidence measures in a chronic disease.

White circles are live coral colonies; green circles are diseased colonies documented during the previous time period (prevalence), red circles are new cases of disease (incidence), and black circles are dead colonies (mortality).



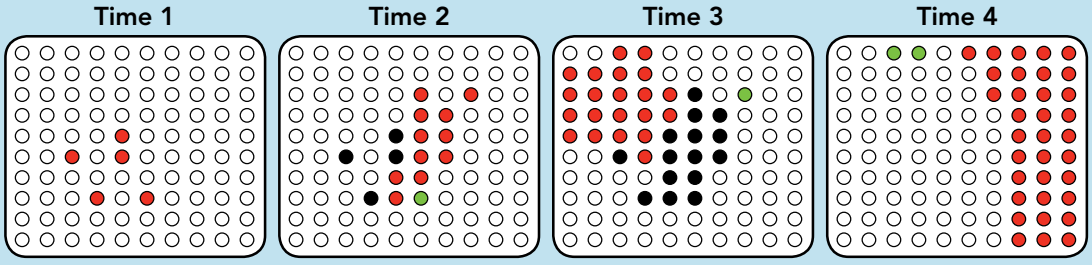
Time	Total cases	New cases	Population	Prevalence	Incidence
1	5		100	0.05	
2	8	3	100	0.08	0.03
3	14	6	100	0.14	0.06
4	24	10	100	0.24	0.1

Example 1: Incidence is much lower over time than prevalence, but increases steadily. This indicates the spread of disease in the population.



Time	Total cases	New cases	Population	Prevalence	Incidence
1	5		100	0.05	
2	8	3	100	0.08	0.03
3	14	5	100	0.14	0.05
4	26	2	100	0.16	0.02

Example 2: Incidence initially rises, reflecting increasing new cases. Incidence then decreases, reflecting a decline in new cases. Prevalence continues to increase the entire time.



Time	Total cases	New cases	Population	Prevalence	Incidence
1	5		100	0.05	
2	12	11	96	0.12	0.11
3	22	21	85	0.25	0.25
4	35	33	66	0.53	0.5

Example 3: An acute lethal disease spreading quickly through a coral population. Incidence tracks prevalence much more closely. The case fatality rate at times 2,3 and 4 would be 80% (4/5), 90% (10/11), and 90% (19/21), respectively. This is very high.

3.2 Basic methods for confirming field assessments and describing disease states

3.2.1 Recognizing disease in the field

This discussion builds on the information presented in **Chapter 2**, and is meant to provide you with more decision-making power. The most critical aspect of recognizing disease states in corals is to "know your animal". While this recommendation may seem trite, recognizing what comprises "normal variation" in morphologic appearance of a coral is a critical first step to understanding the role that disease plays in a reef ecosystem. For example, some coral species have different morphologies or color schemes depending on their geographic location or reef zone. White discoloration is common in the growing tips of some coral species where tissues have yet to be colonized by zooxanthellae, and this could be misinterpreted as partial bleaching (**Box 3.2**).

For the field biologist, diseases in corals are manifested as a change in morphology (via a lesion). When encountering a diseased coral, two important points should be kept foremost in mind:

1. Disease is a continuum between health and death, and this continuum is reflected by changes in morphology as disease progresses. Thus, when observing a lesion on a coral, remember that this lesion could be in the early, middle, or late stages of the disease. Establishing this requires diseased colonies to be marked and monitored over time (see **Section 3.1**, Case Fatality Rate, for methods). Marking colonies with lesions and documenting the progression of the lesion over time can provide invaluable data for understanding how a disease impacts host colonies, how fast it kills tissue, and whether or not colonies can recover or halt the spread of disease.
2. The causes of most coral diseases are unknown, and except for certain cases (see **Section 3.3**), you will not be able to determine the cause of a lesion without further laboratory investigations.

Figure 3.4 A colony of *Porites cylindrica* exhibiting subacute tissue loss from white syndrome. Photo: L. Raymundo

Given these two limitations, the first step to describing a coral disease is to formulate a good morphologic description of the lesion. Doing this is critical for two reasons. First, it forces you to focus on the evidence (i.e. the lesion) and not the potential cause of the lesion. Second, a good morphologic description of the lesion provides the best tangible objective data regarding that disease (pending further laboratory work). These objective data can then be used to communicate facts about this disease to others in a standardized manner, thereby allowing for accurate comparisons of disease among geographic regions. The decision tree in **Chapter 2** (**Figure 2.1**) of this manual will help you do this.

3.2.2 Describing a lesion in a hard coral

Hard corals are relatively simple animals that consist of a thin layer of tissue overlying a calcium carbonate skeleton. Accordingly, a lesion in corals will manifest in three ways:

1. Tissue loss

Tissue is missing, revealing underlying skeleton (**Figure 3.4**). In such lesions, close attention should be paid to the skeleton as this may give clues to the progression or potential cause of the disease. For example, a defined area of bare, intact white skeleton bordered by tissue indicates that tissue loss was recent and relatively rapid (acute). In contrast, a progression from healthy tissue to a band of bare, white, intact skeleton to algae-covered skeleton would suggest an advancing front of tissue loss (subacute). If tissue loss is acute, note whether the skeleton is intact or eroded; acute

tissue loss with skeletal erosion suggests trauma (i.e. fish bite, anchor damage, etc.), and a visual assessment of the immediate area may provide further clues as to the origin of that trauma. When observing white skeleton on a coral, it is helpful to look closely to make sure no tissue is overlying the area; magnifying lenses are helpful for this. Failure to do so may confuse tissue loss with white discoloration (bleaching).

2. Discoloration

This is a deviation from the "normal" color of tissues. The discoloration most familiar to many biologists is white discoloration (bleaching) due to loss of zooxanthellae from tissues. However, other types exist and it is important to reiterate here that many coral species show broad ranges in normal coloration. This must be considered when diagnosing a potential disease state (**Figure 3.5**). When describing the discoloration of corals, it is important to stick with basic colors (i.e. white, purple, pink, brown, etc.) and avoid obscure terms.

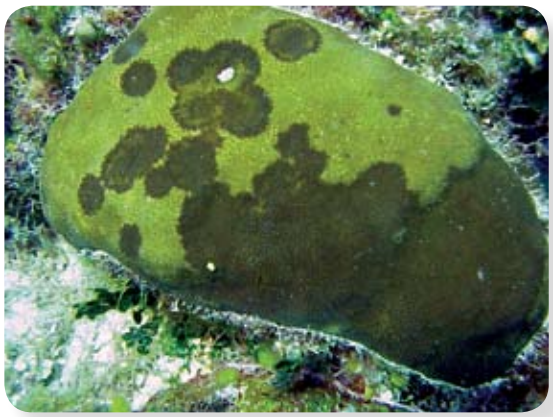


Figure 3.5 Tissue discolouration caused by dark spots disease on *Stephanocoenia*. Photo: E. Weil

3. Growth anomaly

This is an abnormal configuration of the coral skeleton. Typical growth anomalies form as nodular or cauliflower-shaped growths of the coral skeleton. These often lack, or have reduced numbers of, polyps and are discolored white or are distinctly paler in color than surrounding healthy tissue. Other corallilte structural irregularities may also be present, but require a microscope to see (**Figure 3.6**).

Figure 3.6 Growth anomalies on *Goniastrea edwardsi*. Photo: D. Burdick

The three basic types of lesions described above are not mutually exclusive and can occur singly or in various combinations. A final step to describing a coral lesion is to note the distribution of that lesion on the colony. A lesion can be focal (a single occurrence on the colony), multifocal (several scattered or clumped occurrences), or diffuse (encompassing more than 25 percent of the colony surface – **Figure 3.7**). Additional terms and details to systematically describe lesions in corals are available in **Chapter 2**.

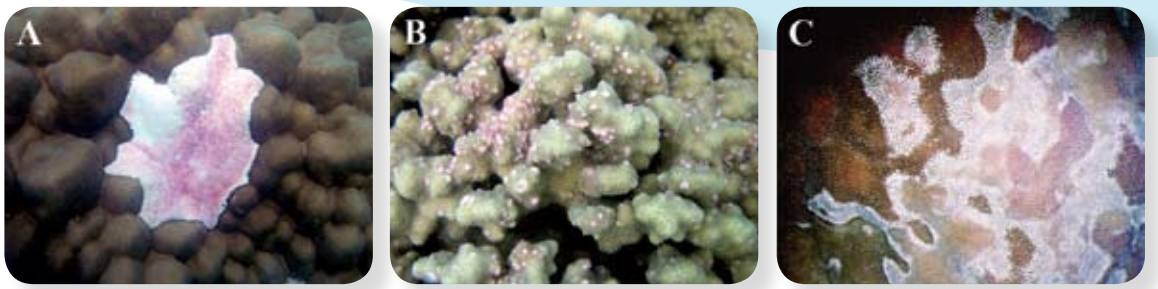


Figure 3.7 Lesion types: (A) focal lesion of a growth anomaly. Photo: D. Burdick; (B) multifocal lesions of trematodiasis. Photo: G. Aeby; (C) diffuse lesion of white syndrome. Photo: L. Raymundo

3.3 Determining causes of lesions: How to collect, handle and preserve specimens for histology, microbiology and molecular assays

3.3.1 The challenge of determining causation

The goal of this chapter is to provide a summary of current practices employed to determine causation of unknown lesions in corals. As we discussed in Chapter 2, determining the cause of lesions from predation or certain environmental impacts may be relatively straightforward if the evidence is present and visible in the immediate environment of the reef. Therefore, an initial assessment of the immediate area surrounding the coral is essential for such diagnoses. For example, a coral displaying 'multifocal acute tissue loss with skeleton erosion in a consistent pattern (i.e. linear, elliptical), and with corallivorous fish nearby inducing similar lesions' should be diagnosed as fish bite (predation). Similarly, 'focal acute tissue loss and erosion of skeleton bordered by a thin band of pink discoloration with presence of snails' could be attributed to snail predation. Other animals may also be visible within the coral skeleton. For example, barnacles will encrust in coral skeleton and be bordered by a thin margin of white discolored tissue, but will be visible in the center of the lesion upon close scrutiny.

In most cases, however, determining the actual cause of a coral lesion is not possible without additional laboratory investigations, particularly if involvement of a microbial agent is suspected. While we recognize a full-on investigation into causation may be beyond the capacity of many managers reading this book, we feel that it is appropriate to provide background information that outlines what can be done, should this course of action be deemed appropriate. Even if it is not within the capacity of a field station, local laboratory, or managing organization to take on such work, we believe it is important to convey the complex nature of investigating coral disease and determining causation. To this end, we offer information on current approaches to assessing and testing for causation. Briefly, this usually involves characterizing the lesion at tissue and cellular levels (histopathology). If an infectious agent is suspected as a cause of the lesion, additional samples may be needed for microbiology (see **Section 3.4** for sampling protocols). In the end, disease investigations in corals are best done through a partnership between coral biologists and disease diagnosticians familiar with appropriate laboratory methods (**Figure 3.8**).

Figure 3.8. Collaboration with local scientists to assist with microbiological analyses is important. Here students are processing samples from coral mucus. Photo: T. Lewis

Determining a specific cause of a disease is, of course, of paramount importance and is a major goal in all disease characterization efforts. However, it is a challenging and lengthy process. If an infectious agent is suspected, one way to address this is by employing a step-by-step process to prove what is known as Koch's Postulates (66). The first step in this process requires collecting diseased and healthy tissue samples and describing the morphology of the lesion at the gross and cellular level which, in some cases, may reveal the presence of a potential causative agent. Various appropriate laboratory tools are used to culture, isolate and identify suspected causative agents. These putative pathogens are then introduced to healthy host tissue under controlled experimental conditions and the response of the host tissue is observed. If lesions develop, the tissue is sampled to re-isolate the introduced microbe. If it is re-isolated from the diseased lesion, then it can be classified as a causal agent for the disease.

Koch's Postulates have been proved for only a handful of coral diseases to date (see **Chapter 1**, **Figure 1.4**), principally due to the limitations of this method. Black band disease, for instance, is the most comprehensively studied disease, but is caused by a microbial consortium dominated by cyanobacteria, *Beggiatoa* and *Desulfovibrio*, which collectively have harmful effects on coral tissue and contribute to its death (67,68). Using Koch's Postulates to prove causation is not an appropriate method in this case, as more than one agent is involved. In addition, certain diseases may be caused by microbial agents that are not culturable, and so cannot be tested using this method. This is one reason why the histological, microbial, and molecular characterization for most diseases remains very incomplete and why alternative methods are currently being developed. Below, we provide some guidelines for collecting and handling specimens to assist in this characterization. It should be stressed that such work requires collaboration between those in the field (i.e. ecologists and reef managers) and laboratory scientists (i.e. histologists, microbiologists, toxicologists, molecular and cellular biologists). Please refer to **Appendix 2** for a list of experts in the field who can be contacted should you be interested in pursuing this route. Arrangements should be made prior to sampling and specimen preparation, so the scientist or laboratory receiving specimens can be prepared to process them.

Figure 3.9. Diver collecting coral mucus with a syringe from the surface of a *Siderastrea siderea* colony. Photo: B. Seymour

Specimens are samples containing material from a disease site, collected for laboratory analyses. Relevant samples include coral tissue (fragments from diseased and healthy tissue of the same colony, as well as from an unaffected reference colony), coral surface mucus and water (collected using a sterile syringe), and sediment (collected with a sterile tube), as well as other flora or fauna associated with the diseased corals (**Figure 3.9**). Available historical or background information surrounding the problem such as that discussed in Section 3.1, along with photo documentation, will assist in the diagnostic process and should be included with the samples (69,70). The specimens must be handled in a manner that preserves the individual sample's identity, prevents cross-contamination and avoids damage to the sample. The preservation method used will be dictated by the intended analysis (i.e. histology, microbiology, toxicology, virology).

3.3.2 General considerations

Permits

Permits for collection and/or transport are required for most studies involving coral disease, regardless of the jurisdiction. The number and type of permits required vary from one location to another. Managers are often in charge of issuing permits, and thus play a crucial role. It is often the manager who must set restrictions on sample size, type and number, and who may be instrumental in establishing procedures for biocontainment, quarantine, or reef closures. Therefore it is essential that collection permit requests include a clear rationale for the type and number of samples being requested.

Safety

The primary consideration when collecting diseased tissue of any kind is personal safety and infection control for both human and coral. Here are general considerations for minimizing risk of spreading disease:

- When multiple sites are to be visited, ALWAYS visit the healthy (or apparently healthy) sites before entering an area with known disease, to prevent potential spread of infectious agents. Similarly, when sampling within a diseased site, first sample the apparently unaffected (healthy) individuals, followed by portions of a diseased colony that appear unaffected (healthy) and sample the diseased portion (lesions) of the colony last.
- Each collected sample should be placed into a separate labelled container; live or frozen tissues should be transported in double containment to prevent any possible contamination back to the ocean at new sites.

- Divers should consider themselves and their equipment as potential vectors of disease between locations. If sampling from multiple reefs, ensure that between sites you quickly rinse SCUBA gear and other equipment in five percent bleach solution (or other suitable disinfectant solution) then rinse in fresh water.

3.3.3 Specimen documentation

Labelling

Label collection containers prior to sampling. A small tag of waterproof paper with information written in pencil can be placed in the container (bag/jar/tube) and allows you to change or add information after sampling. Label syringes for mucus collection prior to the dive as well. Basic label information should include the following:

- collection site
- coral genus and species (if known)
- location on coral where sample was collected (reference tissue from healthy colony, unaffected portion of diseased colony, diseased tissue margin, disease mat)
- date
- initials of sampler

Photographing

Photographing lesions provides a record of color, location and appearance. Both actual size and macro shots should be taken before and after removal of tissue biopsies; include a scale of some sort in each picture (ruler, dive knife, coin, etc). Make sure to document where and when the photo was taken. Photos of the general reef site are useful additional documentation.

3.3.4 Sampling

For Histology

Histological analyses characterize the microscopic morphology of tissue and may help guide further investigations (71). Typically, samples are viewed with a light microscope for general tissue organization. Histological data can reveal cellular changes that occur in tissues under normal, stressed or diseased conditions, and whether foreign organisms (i.e. bacteria, fungi, parasites) are present (**Figure 3.10**).

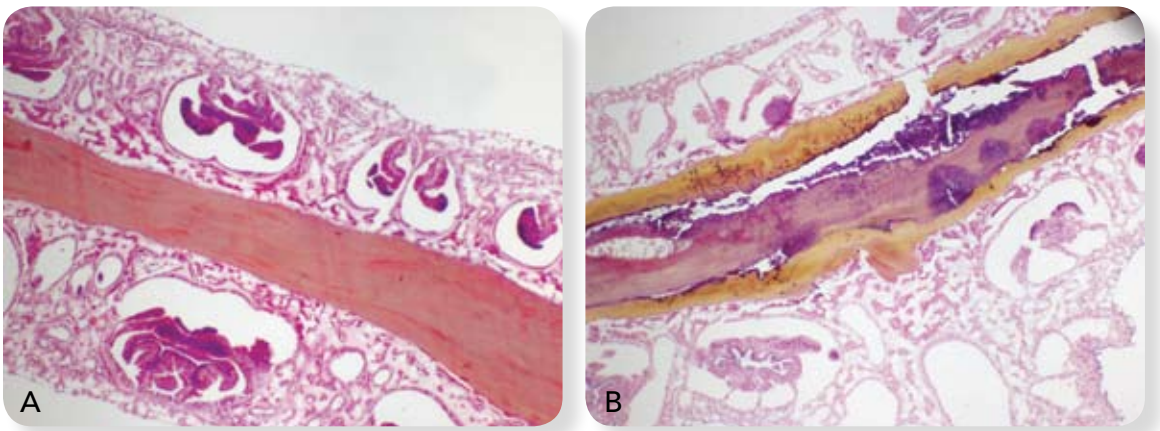


Figure 3.10 Histological samples of sea fan coral *Gorgonia ventalina*. **(A)** tissue from a healthy coral. **(B)** tissue from a lesion of a coral with Aspergillosis; the fungus has spread throughout the skeleton of this coral. This analysis can be useful for confirming field assessment. Photo: C. Couch

Sampling for histology is straightforward, but involves handling potentially hazardous chemicals, so the sampler needs to be aware of proper procedures. In general, a tissue sample is taken and placed in a suitable fixative or preservative. If the analysis is solely for morphology, then 10 percent seawater-buffered formalin is sufficient. However, if immunological or DNA-based staining or preparation for electron microscopy is needed, additional fixatives and procedures are necessary. These preparations should be done under supervision by personnel from the laboratory where the samples will be sent for analysis.

Histological coral samples can be removed in a number of ways. The most simple is removing a small fragment of the coral using a hammer and chisel, coring devices (i.e. leather punch, pneumatic drill, hand drill), rongeurs, wire cutters, or garden clippers. Light microscopy usually requires approximately two cm^2 of apparently healthy tissue taken several centimeters from the diseased tissue and another sample that includes the disease margin (i.e. bare skeleton and intact, diseased tissue). The samples should be placed in labelled plastic bags with fresh seawater until returning to the boat. It is important to completely immerse the sample in fixative as soon after collection as possible (the recommended ratio of fixative to sample is 10:1), noting the time interval between collection and fixation; cellular changes can begin as soon as a sample is removed from the coral.

For microbiological analyses

Microbiological analyses are important diagnostic tools if an infectious agent is suspected as a cause of disease. Two approaches are currently used to isolate and identify possible microbes involved in disease: culture-dependent methods and culture-independent (DNA-based) techniques. Culture-dependent techniques (**Figure 3.11A**) are used to isolate, grow and identify microbes such as bacteria or fungi from lesions, tissue, mucus or surrounding water or sediment. While these methods are useful, they are only able to identify a small fraction of the total microbial community, as many such organisms are not culturable. Therefore, culture-independent methods (**Figure 3.11B**) have been developed that use DNA or RNA present in the samples to examine the diversity of microbial communities in samples or in specific molecular tests (such as polymerase chain reaction assays). Samples are usually taken from healthy and/or diseased coral tissue and coral mucus, to look for shifts in these communities between hosts, healthy versus diseased tissue, species, seasons, geographic location or environments.

The viability of bacteria and their community structure within samples change rapidly and unpredictably after sampling, thus it is critical that samples be processed rapidly. There are two main approaches:

a) sampling specimens for preservation (i.e. DNA extraction, protein analysis, anthropogenic chemical analysis); and

b) sampling specimens that require rapid processing (i.e. live bacterial culture).

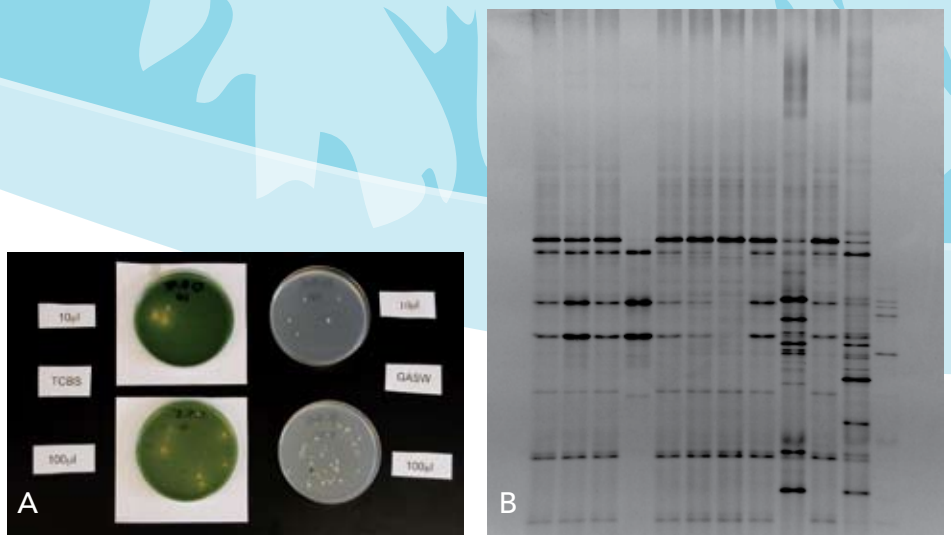


Figure 3.11 Examples of approaches to investigating a potential infectious cause of coral disease. **A)** culture-dependent techniques to isolate and identify microorganisms. The plates shown represent two different culture media (TCBS and GASW) at two concentrations (10µl and 100µl). **B)** culture-independent technique wherein the DNA or RNA of various microorganisms in a sample can be used to identify the number of different organisms present. Photos: C. Woodley

Field work often does not allow for quick processing, so documenting time intervals between sample collection and processing is essential. Samples for culturing live bacteria must be processed within 12 to 24 hours. Although culture-dependent methods are not difficult they do require trained individuals to conduct the procedures. Local hospitals or diagnostic laboratories may be able to assist managers with sampling, plating and culturing techniques. Culture plates should then be sent to a marine microbiologist for further analysis. Some samples, particularly those for international shipments, may require additional permits or documentation for export and/or import, so the collector should be aware of relevant policies and regulations.

Sampling for culture-independent analyses is straightforward but time-sensitive, so while specialized training is not required, attention to detail and proper labelling are essential. As this procedure requires specialized equipment, such work should be undertaken as a collaborative effort between field ecologists and a microbiology laboratory.

Biochemical and molecular analyses

Molecular analyses address the formation, structure, and function of macromolecules such as lipids, metabolites, nucleic acids and proteins. Several such analyses have been adapted to coral and can be used to define a cell's physiological condition or health status (Figure 3.12; 70). The sampling protocol for these analyses is identical to those for collecting tissue biopsies for histology or culture independent analyses, but requires equipment which may not be available. Please consult experts for detailed information.

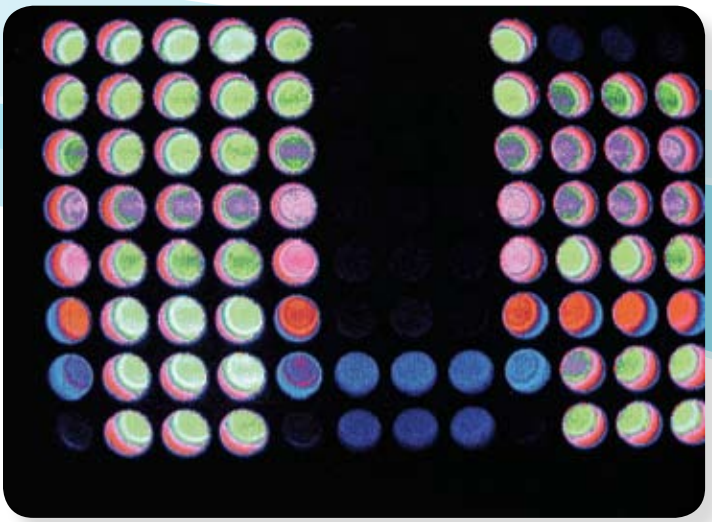


Figure 3.12 ELISA assay for measuring cellular diagnostic parameters. Photo: Haereticus Environmental Laboratory

Specimen shipment

Prior to any sample collection, arrangements must be made with the receiving laboratory(ies) for shipment and analyses. Shipping documentation must be considered, particularly with international shipments, and proper authorization obtained. A key consideration is the protection of sample integrity during shipping. This involves:

- preventing cross-contamination;
- preventing decomposition;
- preventing leakage;
- preserving individual specimen identity; and
- proper labeling (69).

Attention to these will ensure safe delivery and avoid fines (which can be considerable, depending on the infraction). Further regulations and permits (i.e. CITES) may be required depending on the type of sample being collected and shipped, as well as the country of origin and the destination.

Box 3.2

Coral disease nomenclature: a current challenge

Much confusion has resulted from the many reports of new diseases over the last ten years. Several of the names presented in the literature have been assigned on the basis of a few or single observations, and they lack photographic documentation, detail on gross signs, or evidence of coral tissue destruction. Other conditions were presumed to have been caused by a pathogen, but later shown to result from predation or competition. Different researchers have also used terminology interchangeably to describe similar signs, such as the various names given to the white syndromes. There is also a growing number of diseases identified in the Caribbean that have been subdivided (i.e. "Type I" and "Type II"), based on rates or patterns of disease spread or species affected. It can be extremely difficult to verify which "type" of disease is present based on single observations, as initial signs of infection may look different from later stages and rates of spread cannot be determined without monitoring. Furthermore, certain diagnostic features, such as the presence of a bleached front of tissue that advances ahead of the dying tissue, are not readily visible or may be absent depending on the time of observation. For example Caribbean white plague type I and II (6) and white band type I and II (8) differ in rate of progression, a characteristic which is only discernible via monitoring. Dark spot type I and II, dark band syndrome, purple band syndrome and tissue necrosis (11) all basically refer to the same suite of disease signs, as do white pox, patchy necrosis and necrotic patch syndrome (13). The proliferation of names presents difficulties when evaluating host ranges and geographic distribution of coral diseases and can lead to incorrect assumptions about causative agents.

This confusion and lack of coordination has resulted in an increased effort to consolidate information, build consensus regarding nomenclature, and develop the science of coral disease research in a coordinated fashion. Such efforts are vastly improving our current state of knowledge and should continue to do so in the future. Properly describing lesions and disease signs and developing new histological and molecular diagnostic tests is crucial to this effort. It is hoped that the information in this book will further assist in the development of a coordinated approach.

Chapter 4
Assessment and Monitoring Protocols

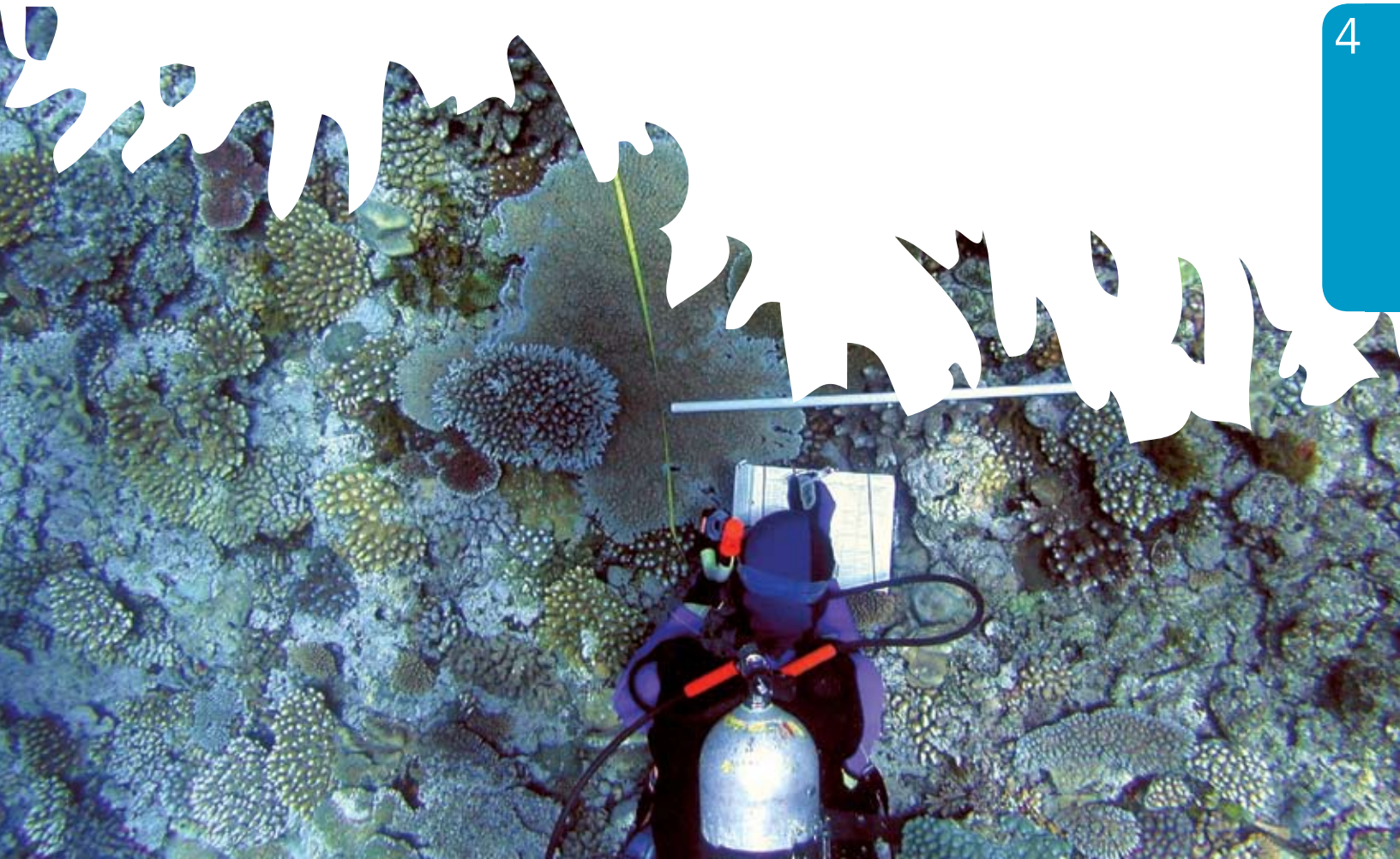
In this chapter you will find:

Objectives and methods for rapid assessment and long-term monitoring.

Designing a valid and reliable sampling protocol.

Quantifying coral disease prevalence, incidence and mortality rate.

Guidelines for measuring basic environmental variables which may impact disease dynamics.



Assessment and Monitoring Protocols

E. Weil, E. Jordán-Dahlgren, A. Bruckner and L. Raymundo

4.1 Rapid assessment versus monitoring – goals and objectives of each

Most protocols used to assess coral reef ecosystems involve characterizing coral cover, species diversity, fish community structure, water quality, and human activities, with an emphasis on detecting change. While this approach may provide a detailed picture of reef condition at the time of the survey, and how the reef has changed between survey periods, it may fail to identify factors responsible for the changes observed, or predict future trends under varying management scenarios. Alternatively, surveys focusing only on a specific threat to a community, such as disease or bleaching, may provide detailed diagnostic information and rigorous data on prevalence, incidence, and impacts, but may miss additional information on reef condition that is critical to understanding the impacts of the threat.

Figure 4.1 An integrated approach to obtain quantitative data on a reef community can provide a more complete picture of a reef's health status than a survey examining only disease states. Here, divers take detailed data on the coral population. Photo: B. Willis

The ideal approach to studying coral diseases and their impacts, given sufficient funding and qualified personnel, is a well-designed, integrated, multi-component survey. Such a survey would provide population, community and/or ecosystem-level data on benthic organisms, fishes, water quality, environmental parameters and the human dimension, along with disease components at different spatial and temporal scales (**Figure 4.1**). This approach allows assessment of coral reef community structure and function, temporal changes, and potential links between assessed parameters that might be responsible for observed changes in reef condition. It is important to understand that significant correlations between disease prevalence and environmental and/or biological factors do not prove causality. It simply suggests associations between the variables considered.

Due to the potential for high coral mortality from disease, estimating the magnitude of disease impacts should be a fundamental management goal. There are two main approaches in current use: **rapid assessments** and **monitoring**.

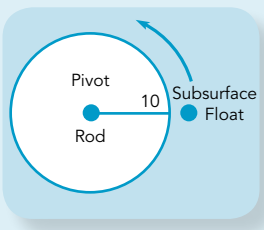
1. **Rapid assessments** – These characterize the reef area(s) surveyed at the moment of the assessment; a single point in time. This usually estimates relatively static (state) variables about a population or a community and is useful for comparing multiple sites or different time periods.

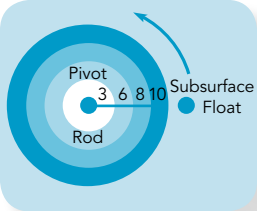
2. **Monitoring** – This detects changes over time within the same reef area(s). The aim of such surveys is to estimate changing (process) variables. For both approaches, a protocol that answers specific questions must be designed. For instance, initial questions for a protocol that seeks to describe a general reef condition would include:

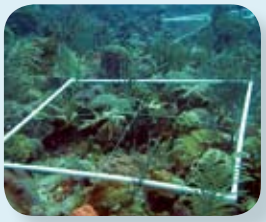
- Are there coral diseases present on the reef? If so, which ones?
- What species are affected?
- Are there reefs, reef zones or reef areas apparently more affected than others?

In this case, a relatively simple sampling effort using qualitative or semi-quantitative rapid assessments could provide the answers (see **Table 4.1** for surveying techniques). On the other hand, monitoring would address the questions of changes over time – something rapid assessments cannot do. Therefore monitoring should be the approach considered in any short or long-term management program for Marine Protected Areas and other areas of high conservation value.

Table 4.1 Descriptions of commonly-used surveying techniques. Descriptions taken from Edmunds (1), Porter & Meier (5), Antonius (7), Kuta & Richardson (9), English et al. (10), Bruckner & Bruckner (12), AGRRA (14), Jaap et al. (19), Santavy et al. (20), Bruckner (21), Weil et al. (25), Feingold (27).

Survey	Description	Advantage	Disadvantage
Manta Tows 	A diver/snorkeler is towed behind a small boat at a slow and constant speed for a fixed time interval.	Allows rapid coverage of large areas. Provides estimates of coral cover, dominant coral types, broad mortality estimates.	Detailed diagnostic or quantitative data not possible. Dependent on high water quality. May only be useful to estimate mortality cause if very visible (i.e. COTS, BBD).
Timed Swims 	Diver swims for a fixed time in a straight line along a single depth gradient. All diseased corals in a 2m band noted: species, disease type, lesion number.	Provides semi-quantitative information on abundance of disease over large areas.	Not used for prevalence or incidence (no total colony count; survey area only estimated). Disease count categories used: rare (1-3 cases), moderate (4-12 cases), frequent (13-25 cases), abundant (26-50 cases), epidemic (51-100 cases), catastrophic (>100 cases)
Circular areas 	Infected and healthy colonies of all species counted within a circular area (10m radius; 314 m^2). A stake pounded into substrate is used to define the pivotal center of the circle; a 10m transect tape is held by diver as she/he swims around the stake, keeping the tape taut.	Provides quantitative data on prevalence and incidence of diseases. If size measurements are included, population structure can be estimated. Best on flat reef substrates, in studies of single diseases (BBD, YBD).	Cannot be used on a reef slope because multiple zones may be included in a single sampling site. Does not provide information on coral cover unless combined with other measures. Impractical in areas with high cover and density.

Survey	Description	Advantage	Disadvantage
Radial belt transect 	Sampling circular areas in concentric belts (or arcs) around a pivot point. The area sampled is either a contiguous circle (radial sampling) or a series of rings (radial belt transect) around the pivot point.	Counts the total number of infected and healthy colonies of each species within the outer 8-10m of a circle (314m^2 plot) and identifies all diseases.	Assessment of multiple permanent sites may be time consuming.
Belt transects 	All corals within a predefined area (i.e. 2x20m) are counted and disease presence recorded. 1m or 2m PVC stick is used to define a quadrat along a transect. May include more measurements such as colony size, coral cover, and percent mortality.	Can provide detailed data on prevalence based on a whole colony assessment, population dynamics, and health status. Long term monitoring of tagged colonies can provide data on colony fate (recovered/ dead/stasis, etc.).	Requires multiple transects in each zone. With high diversity, high cover and abundant small corals, individual transects may require multiple dives to complete.
Line intercept transects 	Can provide detailed data on prevalence based on a whole colony assessment, population dynamics, and health status. Long term monitoring of tagged colonies can provide data on colony fate (recovered/ dead/stasis, etc.).	Allows rapid assessment of coral community structure, condition, and prevalence of disease from a whole colony perspective. Provides information on size structure, colony density, coral cover.	Requires multiple transects throughout each zone to quantify prevalence. Does not provide a comparable assessment of area surveyed if corals vary in size between sites. Colony size based on actual measurements (or size classes) but percent colony mortality is estimated and may vary between observers.
Point intercept transects 	Requires multiple transects in each zone. With high diversity, high cover and abundant small corals, individual transects may require multiple dives to complete.	Provides information on cover of various benthic organisms including coral as well as substrate types. Faster to use than Line intercept, if multiple survey sites are needed; allows rapid assessments.	Prevalence may be incorrectly assessed because relatively small overall area is examined along each transect. Does not provide detailed information on colony abundance (large colonies may be counted twice) or size structure.
Chain transects 	Biotic and abiotic components identified directly under each link in chain. Rugosity estimated by determining ratio of the length of chain laid following bottom contours to the straight-line distance between start and end points of the transect.	Provides rapid information on reef rugosity, species diversity and cover.	Assesses a very narrow band of reef. Diseases and other factors may be missed unless the chain lands on the diseased portion of a colony.

Survey	Description	Advantage	Disadvantage
Quadrats 	Quadrats of various sizes ($0.5m^2$, $1m^2$) are placed haphazardly, randomly, or at specific intervals along transects. Percent cover of all species and substrate types within quadrat area determined by counting number of quadrat subunits occupied by each category.	Provides quantitative information on cover of coral and other organisms and substrates, and qualitative data on types of disease present.	With exception of large quadrats (i.e. 100x250m), poorly estimates disease prevalence, abundance, size or condition of corals; does not capture disease on the portion of a coral that falls outside the quadrat. Does not work well for large thicket-forming corals.
Photo-quadrats 	Quadrats of varying sizes ($<1m^2$) are photographed using high resolution digital cameras and video.	Accurate assessment of cover and changes in cover (when using permanent quads). Less bottom time; data are analyzed in the lab, using image analysis software, such as NIH's free Image J software®.	Requires considerable lab work to analyze images. May fail to detect diseases and small colonies. Does not work well for large branching corals that form thickets. Resolution too low to identify many corals to species.

Validity and reliability

The importance of proper sampling design cannot be underestimated, particularly if the data are going to be applied to management. A good assessment must be both valid and reliable. *Validity* refers to how effectively the assessment reflects what we want it to measure, recognizing that we can only survey a small portion of the entire reef. Adequate sample representation is, therefore, a crucial component. *Reliability* pertains to how efficiently a method measures specified parameters over the entire set of sampling conditions likely to be encountered, and should incorporate valid replicability. Sample representation and replication ensure that statistical power is optimized, a given situation is properly characterized, and meaningful comparisons can be made in space and time.

Standardized sampling protocols

Many current surveys use a standardized sampling protocol. This facilitates regional comparisons but sometimes sacrifices a detailed characterization of the complexity of local reef environments. Therefore, it is important to consider what the objectives of an assessment or monitoring scheme are. If the data are to be used locally to address management issues, then it is important to take into account the complexity of the local reefs. However, if the data are to be deposited into a larger database for regional or global comparisons, then a standardized protocol applied across sites is necessary. With the general deterioration of reef communities at local and regional levels, it is clear that the best approach combines the two.

4.2 Coral disease rapid assessment and monitoring protocols

Validity and reliability of all types of quantitative assessments require satisfying a fundamental condition: *an adequate estimation of the natural variation of the chosen parameters* (*disease prevalence, coral cover, etc.*). Imposed upon this critical point is the very real consideration of time, resources and personnel limitations. The following recommendations are provided to help address these issues.

What are the goals of the sampling?

What questions are to be addressed and at what spatial and temporal scales? Rapid regional assessments can reveal large-scale processes such as the expansion rate of a particular disease from an infection 'hot spot' to nearby reefs, and serve as an early warning system to identify and track disease outbreaks. Monitoring targeted to address very specific questions can provide data on the status of a particular disease or coral species, seasonality, incidence and effects of diseases at a local scale, and the role of localized stressors on disease processes and impacts. Monitoring individual colonies will result in the documentation of patterns of spread, rates of tissue destruction, impact of diseases at a colony or population level, and the fate of affected colonies.

How many sites?

Reefs can generally be divided into distinct zones, created by strong environmental gradients acting on the benthic community. The back reef, reef flat, reef crest, fore reef or slope are the standard zones, though these can be highly modified by bathymetry and coral growth, and may even be absent. Further, depth can change drastically within a zone and provide even more complexity. Here, we define "site" as a zone or habitat of distinct structure within a reef.

For descriptive or rapid assessment surveys, logistics and time constraints may make it necessary to limit sampling to a single zone within a reef (such as the reef crest). In such cases, the same habitat or zone should be surveyed at each location. If great variation exists in community structure within a depth range or zone (due to high relief, spur and groove formations, etc.), there may be more than one "habitat" present and further within-zone stratification will be required. For space/time comparisons, at least three replicate sampling units per zone/habitat (linear transect, radial arc, etc.; see **Table 4.1**) are recommended, as differences between sampling units can only be examined in comparison to variation within sampling units.

What sampling technique and sampling unit?

Consider objectives, logistics, time, and resources, and refer to **Table 4.1** for the most commonly used sampling techniques. Select the technique that best suits the local conditions while addressing the objectives of the sampling. The same sampling technique should be used in all areas. The sampling unit is defined by the question(s) asked, which should consider appropriate spatial and temporal scales. For example, if the question refers to colony properties, the sample unit will be each colony and the technique may involve censusing all colonies within a defined area. If the question refers to populations or communities, then the sampling unit will be an area or length of reef that is surveyed. Belt transects, circular areas, and quadrats are examples of area sample units, while line, chain and point intercept transects are examples of linear sampling units. Sampling units should be replicated within each site; this is discussed in the section below on sampling unit number.

What sampling unit size?

This depends on the size and type of spatial distribution of the coral colonies and, in practice, on logistical constraints. A community with large colonies requires larger sampling areas than one dominated by smaller ones. Area-based sampling units (quadrats, belt transects, etc.) bias count data, due to the effect of including or excluding colonies that extend beyond the edges of the sampling unit (i.e. the "edge effect"). The larger the colony size in relation to the size of the sampling unit, the larger this effect, as a greater proportion of colonies will be excluded. To minimize the edge effect, Green (72) recommends that the ratio of colony size to unit sample size should be very small: a sample area should be 20 times larger than the mean colony size. Zvuloni et al. (73) propose a mid-point criterion: if more than 50 percent of the colony lies within the sampling unit, the colony should be counted. If a reef community contains many single-species stands (i.e. *Acropora* thickets), then a smaller size is

convenient (see Green 72, for details). Again, within a single monitoring program, the same sample unit size should be used in all reefs. This may require some compromise as reef communities are unlikely to be the same, but it is an important point if data are to be compared across reefs.

How many sampling units per zone/site?

Replication is mandatory because without it, there is no way to estimate variance of the measured parameters. For a preliminary sampling effort, three sampling units per site may be enough, provided the site is relatively homogeneous. If resources permit, six sampling units are sufficient in most instances. The number of replicates should be constant for all areas compared, i.e. six belt transects of 20m² (10mx2m) in each of three habitats or depth intervals (deep reef, intermediate depth and shallow reef). This amount of replication (18 total sampling units) adequately samples the different habitats and the reef as a whole, provided the sampling units are adequately distributed. However, this takes a large amount of time and may not be logistically possible. In such cases, it may be necessary to sample at two depths, and to cut the number of sampling units to three per depth.

How should the sampling sites and units be spatially distributed?

Sampling sites and units should be randomly distributed within the reef zone/site area if possible (**Figure 4.2**). If the sampling units are not independent of each other and are somehow influenced by the researcher's own decision-making process, then a major violation of statistical principles has been made and the results will be invalid. Therefore, spatial randomization must be worked out beforehand. The best way to do this is by following these steps:

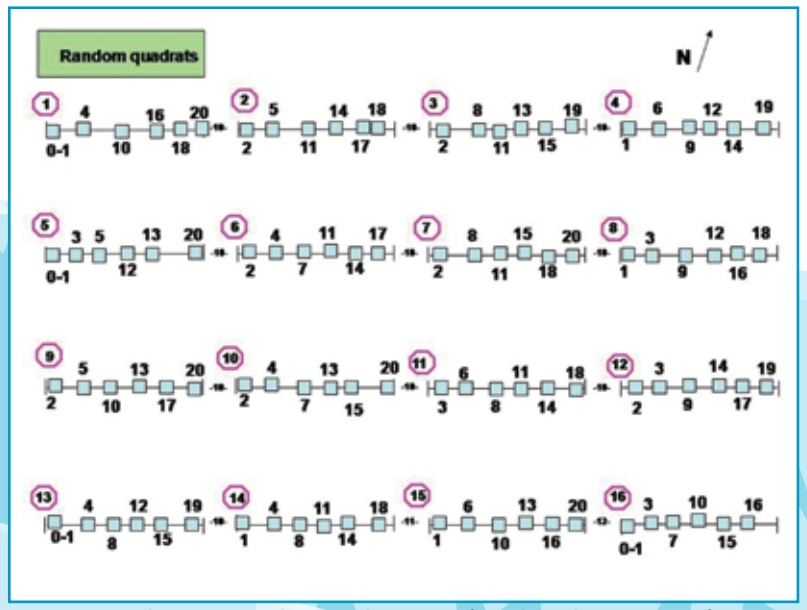


Figure 4.2 A schematic map showing placement of quadrats along a series of transects. The quadrats to be surveyed were randomly selected prior to surveying.

1. If a benthic map is available that shows the different reef zones or habitats, roughly define the spatial extent you are interested in sampling using geographical coordinates.
2. Within that area, overlay a square grid. The squares should be slightly larger than the area of the sampling unit to avoid overlap.
3. Then assign a number to each square, and randomly select which numbered squares will be sampled. (There are several free random number generating programs available on the Internet.)
4. Upon arrival at the reef, find the squares to be surveyed using a GPS. Finding the predetermined squares using GPS at the site is challenging, and will not be possible if you do not have access to a boat. If this approach is not possible, then it is common practice to locate the general reef habitats once at the site, and to place replicate sampling units equidistant from each other while keeping depth constant within a habitat or zone. This can be done by deciding on a predetermined number of fin kicks to swim underwater between transects.

By making an arbitrary decision regarding placement of transects before conducting the surveys, you will avoid observer bias.

Permanent or re-randomized sampling units for monitoring?

Different monitoring programs use different strategies. The decision to establish and resurvey permanent sites or randomly select new sites to survey each time may or may not be within the manager's control. However, when a manager is able to make this decision, the pros and cons of the two approaches are outlined below.

Permanently fixed sampling units

Permanent sampling units minimize variability due to sampling error, which increases data reliability. The main challenge lies in taking the care to establish well-marked permanent units. If this is not done, then searching for underwater markers and re-deploying sampling units can eat up valuable bottom time underwater. There are steps that can be taken however, to make relocation relatively straightforward (see **Box 4.1**). Furthermore, accuracy in relocating permanent sites increases over time as observers become more familiar with the site. Finally, permanent sites allow estimation of disease incidence and impacts over time using many fewer sites. This approach may provide specific information for a particular reef or zone but much more time is required to discern patterns over multiple reef systems.

Re-randomization of sampling units

Re-randomization of sampling units for each survey of the same reef site may result in more comprehensive data on prevalence over an entire reef system than is possible with fixed transects, as a greater reef area is surveyed over time. However, variance is likely to be higher due to micro-habitat differences and sampling error, and a larger number of sampling units will be required to achieve the same statistical power. Furthermore, the number of random sites needed to measure change will be much greater because you are not looking at the same corals each time.

The optimal approach, given sufficient personnel and funding, is to establish permanent sites in representative habitats/depths and supplement these with a larger number of random sites that are examined using a rapid assessment technique.

What sampling frequency?

It is preferable to sample quarterly or more often to avoid missing disease outbreaks and capture any seasonal component that might be impacting the reef. Diseases often affect individual hosts for a relatively short time, with new infections appearing on surrounding corals at frequent intervals. Infrequent sampling makes it difficult, if not impossible, to accurately assess the duration of individual infections and their role in coral mortality. Further, targeted samplings during a certain time of year may incorrectly estimate the importance of disease in structuring coral communities. Coral mortality may be caused by numerous factors in addition to disease, which may not be recorded if samplings are conducted infrequently. Because outbreaks caused by rapidly-progressing diseases may die out in a short period of time, it is advisable to conduct rapid assessments frequently and be ready to conduct quantitative surveys if such an event occurs (see **Chapter 5**, for details on characterizing an outbreak).

When are controls necessary?

In scientific research, a "control" is the treatment whereby all parameters are exactly the same as for the experimental manipulation except for the one parameter that is being tested. This allows a comparison of the effect of the parameter. In ecological monitoring, control sites may be necessary if the objective is to examine the effect of an impact on some aspect of the reef community. In this case, a control site is one unaffected by the impact and against which impacted reefs are compared. For example, if the objective of a monitoring program is to quantify the effects of a sewage outfall on disease prevalence, it is necessary to identify a minimum of three replicate sites predicted to be affected by sewage. The same number of sites remote from the outfall, but of similar structure, must be selected as un-impacted controls against which to compare the effect of sewage.

Why is preliminary sampling advisable?

Prior to beginning a major rapid assessment or monitoring effort, it may be necessary to conduct preliminary pilot assessments. Such assessments are useful in estimating the amount of variation in the parameters being monitored (i.e. live coral cover, prevalence, etc.), so appropriate decisions can be made regarding replication and sampling technique. Preliminary sampling also has an additional, rather practical benefit – most potential problems can be identified early. These include problems relating to sampling design decisions (sites, stratification, type, number and size of sampling units, taxonomic level desired, disease types or signs) and field logistics. Therefore, the final sampling design will be based on direct, practical knowledge of the area, rather than on untested assumptions.

4.3 Designing a monitoring program

A comprehensive coral disease monitoring program should apply principles of **epidemiology** and risk analysis to coral health assessments. This will help identify predictors (i.e. risk factors) for changes in coral and ecosystem health (such as warm temperatures or increased nutrient loads); quantify the strength of those associations; and focus diagnostic efforts towards identifying etiology (see **Chapter 3**). Standardized disease monitoring programs can be supplemented with the following:

a. detailed disease assessments (see **Appendix 4** for sample data sheets);

b. additional population information (i.e. abundances, size classes, species diversity, and health status);

c. quantification of species which may indicate potential health risks to corals, or improvement in reef health as a consequence of management (i.e. macroalgae, herbivores, coral predators); and

d. measures of water quality (i.e. sedimentation, nutrient input, bacterial load). Often, water quality monitoring may be undertaken by a different local agency or individual, so collaborative agreements between coral disease and water quality monitoring agencies or individuals may result in reduced cost and effort.

Monitoring should be proactive; it can help predict the likelihood of events such as a disease outbreak, responsive changes in community structure, and recovery rates. A combination of rapid random assessments and long-term monitoring of permanent sites provides the most comprehensive picture of the impacts of disease on an area.

4.4 Characterizing long-term disease effects on population dynamics, community structure and ecosystem function

Monitoring permanent sites (in addition to random surveys) results in data on the rate of occurrence of new infections and disease impacts at different scales (colonies, species, populations, communities). Long-term monitoring of individually tagged colonies affected by disease can also provide detailed information on the spatial and temporal dynamics of particular diseases. Such information might include:

- the rate of spread of infection within a colony;
- amount and patterns of mortality sustained from an infection;
- change in the number of lesions over time;
- change in lesion spatial distribution;
- lesion dynamics (position of lesions on colony, temporal scale);
- duration of infection;
- host colony fate (recovery, progression, stasis, mortality, re-infection); and
- environmental correlates.

A monitoring program implemented for coral diseases at the population/community level should address the following:

- types of diseases/syndromes, host range, and variation in disease dynamics between species;
- prevalence and incidence of diseases (population or species level), duration of an outbreak, consequences to host species, and variability at various spatial and temporal scales;
- local, regional and global distribution of diseases;
- physico-chemical parameters (depth, water clarity, temperature, nutrient load, etc.), biological factors (predators, algal abundance, bacterial load, etc.), and anthropogenic impacts (pollution, runoff, sedimentation, etc.) associated with either chronic or acute disease events;
- long-term effects of disease on coral reef community structure and function at local and regional scales; and
- potential reservoirs and vectors of disease.

4.5 Establishing and relocating permanent monitoring sites

Working with permanent sampling units can be challenging to begin with, as it involves finding an original marker position after time has lapsed, doing so in changing environmental conditions, finding markers that are covered with overgrowth, and working with different personnel. However, it is important to do these things properly so the time spent setting up permanent sites does not go to waste.

Once sites are selected and the survey method defined using the guidelines in Section 4.2, a list of materials must be produced (see **Table 4.2**). This list should include everything needed for:

a) marking and finding the sites;

b) marking and finding the permanent sampling units; and

c) collecting data.

Many of these materials should be available during every survey because re-bars, tags, flagging tape, buoys, etc. can disappear between surveys and require replacement.

When establishing permanent sites, researchers should attempt to minimize human interaction on the reef and deploy permanent stakes and tags in as discriminate a manner as possible. For example, re-bars and masonry nails should never be inserted into live coral and all floats, cable ties, tags and other materials should be secured such that they do not abrade corals or other sessile invertebrates. A procedure for establishing permanent transects is described below. This method requires insertion of multiple re-bars per transect. Other approaches, such as those for radial or circular sites, require a single permanent rebar (and submerged buoy or float to facilitate relocation of the site) and equipment for subdividing the site into measurable units, which are temporarily deployed only when assessments are underway. After the study is completed, the markers should be removed.

Table 4.2. Recommended materials list for setting up a permanent survey area within a reef

Site ID materials	Marking materials	Data collection materials
Differential GPS	1/2 m long 5/8' re-bar stakes	UW disease ID cards
Depth gauge	Heavy hammer or mallet	UW coral species ID cards
Mooring buoy	Large plastic numbered tags	Slateboards
Rope	Cable ties (different lengths)	Plastified UW paper
UW digital camera	Small floaters/buoys	Transect tapes
Temperature loggers*	Monofilament for tying	Mechanical pencils
Submerged buoy(s)**	Clipper and dive knife	Underwater notebooks
	Masonry nails	Magnifying lens
	Marine epoxy or Z-spar®	Plastic caliper or ruler
		Plastic bags+
		Hammer, chisel, wire cutters

*HOBO® temperature loggers are easy to deploy and can remain at a station for up to five years.**At some sites, it may be necessary to deploy marker buoys underwater. A GPS unit can be used from the surface to mark the site of a submerged buoy. +Plastic bags are used to store diseased tissue sampled using a hammer and chisel or wire cutters, if a microscope is necessary to verify field determination.

Establishing a permanent monitoring site using a transect

Figure 4.3 A rebar stake used to position a transect line. Photo: C. Caballes

Figure 4.4 Laying a transect line along a permanently monitored site. The line must be laid taut, so that the area monitored remains the same over time. Photo: C. Caballes

Refer to **Box 4.1** at the end of this chapter for advice on how to relocate sites in successive visits. For the regional assessment and monitoring of diseases in the Caribbean, for example, the design included five 10mx2m (20m²) belt transects in each of three depth intervals (habitats) per reef (n=15 per reef, with a total surveyed area of 300m²), three reefs in each country, and three countries per major geographic region (Weil, pers. comm.) Below is a step-by-step procedure for establishing a permanent monitoring site using a transect:

1. If the sampling design includes depth intervals or multiple reef habitats, start with the deepest habitat first. Once a pre-survey swim of the site has revealed the optimum general area for monitoring, hammer in the first tagged re-bar and deploy the transect tape, keeping depth relatively constant (**Figure 4.3**). At the end of the predetermined transect length, hammer in the end re-bar. Re-bar stakes may require additional epoxy, cable ties, or other means of stabilization; they must be completely immobilized and secured to the bottom. The first re-bar of each transect should already have the tag number and/or buoy attached so you do not waste time underwater. Use plastic tags with large numbers (such as livestock tags; see **Figure 3.3**).
2. Hammer in masonry nails every 5m along the tape, avoiding living tissue. These will allow placement of the tape over the same line every time. If there is surge or currents, the tape is wound around the nails to prevent bending and displacement. In reefs with high topographic relief or on transects more than 10m long, nails may not be visible; re-bar stakes can be used in their place at 5-10m distances along the transect length. The transect tape should be laid along this line tautly, to prevent displacement by surge or current (**Figure 4.4**).

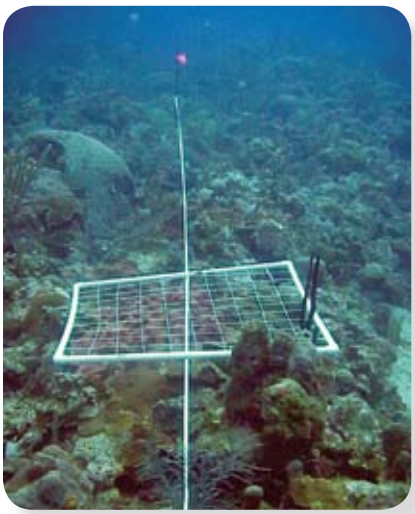


Figure 4.5 Flagging tape tied to the end of a re-bar stake provides a visual marker for relocating a transect. Photo: E. Weil

3. Tie flagging tape or a submerged buoy onto the beginning and end re-bars of the transect (**Figure 4.5**). An additional buoy/floater can be tied to the midway transect marker (nail or re-bar). This will facilitate finding the transect in future surveys.
4. To place the next belt transect haphazardly, use a pre-established method such as moving 1m to the right or left of your previous transect and swimming 10 fin kicks. Then, hammer in the first re-bar of the second transect. Repeat this procedure with all transects at all depths. If quadrats are used along the transect, their position should be randomized (see **Figure 4.2**) and recorded. Rebar stakes or nails can be used as reference points to position the quadrat in exactly the same position each time (**Figure 4.5**).

4.6 Calculating prevalence, incidence, and gross disease characteristics

Data collection

Data from any sample unit that surveys a defined area (belts, quadrats, circles) may be applied to the calculations detailed below. Using the selected sample unit, all colonies larger than 5cm in diameter (or whatever criterion is selected) are counted, identified to whatever taxonomic unit is decided upon (species, genus, morphological type, etc.), and examined for presence of disease or compromised health, using categories outlined in **Chapter 2**. These data are entered on a data sheet (see Appendix 5 for examples in current use). How precise a taxonomic identification is needed depends on the questions, surveyors and geographic area. In the Caribbean, there are only ~62 zooxanthellate coral species, so collecting data at the species level is possible. In the Indo-Pacific, more than 700 species have been recorded, so data are normally collected at the genus level, and segregated by morphology (branching, massive, encrusting, etc.). Additional data on colony size and percent mortality can provide useful information on population dynamics, community structure and impacts of disease and other stressors. For example, in the western Atlantic many researchers measure the height and diameter of colonies and record the percent of the colony surface with recently denuded skeleton and older, algal colonized skeleton.

Disease prevalence

Disease prevalence is the proportion of diseased colonies to the total measured population of colonies. It can be calculated for individual populations, species or genera, or for the coral community as a whole, as well as for each particular disease/syndrome, similar group of diseases or for all diseases lumped together. What is calculated depends on the questions asked.

$$\text{Prevalence (P)} = (\#\ \text{diseased colonies} / \text{total}\ \#\ \text{of colonies}) \times 100$$

A prevalence value is estimated for each area-sample unit. An average prevalence value with standard deviation can then be calculated for habitats, zones or reefs (depending on the stratification and the questions) using the sample unit prevalence value.

Disease incidence

Disease incidence is the number of new infections appearing in the population within a period of time. This is a very important record of the progress of a particular disease in a particular species, population or community; it characterizes the epizootic temporal dynamics. To estimate the incidence of a particular disease, all infected colonies found during the first survey should be tagged and/or mapped within each sampling area. This is particularly important if the disease is short-lived or seasonal (such as black band disease or white plague). During subsequent surveys of the same sampling units, newly infected colonies are then identified, counted, tagged and mapped. Dead colonies should be counted and caution must be taken to make sure colonies are not counted twice. For example, if a colony is infected during time T_2, and is dead at time T_4, it is only added to incidence calculations at time T_2. Records of dead colonies should be noted separately, as mortality rate. However, if the colony was healthy (and, therefore, unmapped and untagged) during a previous survey (i.e. time T_2), but dead by the next survey period (i.e. time T_3) then it should be counted in both incidence and mortality calculations at time T_3 (but only if cause of death can be verified as the disease in question). It should also be tagged and mapped so that it is not recounted in later surveys. Average incidence is calculated for the site as a whole after incidence values are calculated for each sampling unit for the habitat and/or reef in question. See **Chapter 5** for how to use incidence observations to calculate rate of outbreak.

$$\text{Incidence (I)} = \text{number of new infections within a time period, T}$$

Mortality

Often, the aspect of most concern is mortality rate, as this can have profound consequences on the structure of a reef community. Mortality rate can be calculated as follows:

$$M = \frac{\text{number of colonies dying per census area per unit time}}{\text{total number of colonies within census area}}$$

However, if the dynamics of a particular disease are of interest, such as how this disease is affecting a particular species, or how severe it is, then the case fatality rate (**Chapter 3, Section 3.1**) may be of interest. The case fatality rate measures the mortality rate of those susceptible and affected by a particular disease:

$$CF = \frac{\text{number of colonies dying of a disease per census area per unit time}}{\text{total number of colonies with the disease per census area per unit time}}$$

These calculations can be used on individual coral species, or on the coral population as a whole.

Another aspect that may be of interest is partial mortality. Corals, as colonial animals, can "partially die", i.e. they may lose a certain portion of their tissue, but the remaining tissue may be healthy and capable of regrowth. Therefore, it may be of value to monitor partial mortality as a percentage of the colony which dies as a result of a disease. One can then monitor whether or not the tissue regrows and recovers after partial mortality has apparently stopped, whether partial mortality continues to progress to full colony mortality, or whether the disease appears to come to a halt, but with no apparent tissue regrowth over bare skeleton. In these cases, it is necessary to develop a way of determining the percent of the colony surface area affected. For branching and foliose morphologies, the total number of branches, as well as dead and dying ones, can be counted and a 'percent of colony affected' calculated. For massive, plating and encrusting forms, the percent of affected surface area can be calculated from photographs, (see "using photographs" below), or lesion and colony diameter can be measured by hand using a transect tape or calipers. If hand measurements are to be used it is customary to take a measurement at the widest diameter and then one perpendicular to the widest. The mean can then be calculated from these two, and the 'percent of colony affected' calculated as the ratio of lesion size to colony size.

Disease characteristics at the colony level

If colonies are tagged in order to monitor disease characteristics at the colony level over time, representatives of both diseased and healthy colonies must be tagged within or near the sampling unit. If the disease is affecting multiple species, then replicate colonies of each species should be monitored. Logistics and time resources should be considered when deciding how many replicates to tag, although five healthy and five diseased colonies per species would be a minimum number. The information obtained through this process (such as that listed in **Section 4.4**) is useful for characterizing the etiology of particular diseases. These diagnostic characteristics help define the etiology, such as daily, weekly, monthly or seasonal changes in lesion or band appearance. All information should be collected in a template sheet already formatted for this purpose to reduce the amount of underwater time needed, and to standardize data collection.

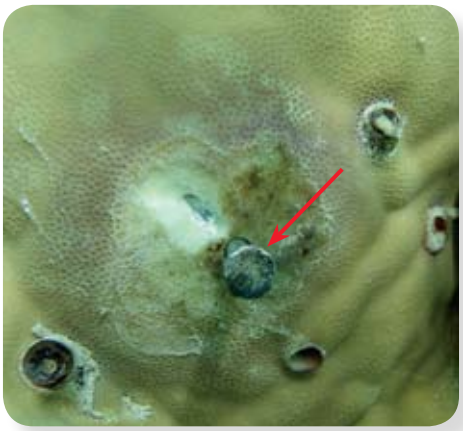


Figure 4.6 A masonry nail (see arrow) hammered into dead skeleton can provide a useful reference point and scale for monitoring disease progression in an active lesion. Here a massive *Porites* colony exhibits signs of white syndrome. Photo: L. Raymundo

Using photographs

Photographs of diseased colonies are important sources of information. When taking photos for later analysis, always place an appropriate scale bar in each photo; a plastic ruler is the easiest to use. In addition, in massive and encrusting morphologies, a masonry nail can be hammered into the colony at the immediate edge of the advancing front when the colony is initially tagged (hammer into dead skeleton, not into live tissue; **Figure 4.6**).

Figure 4.7 A branch of *Acropora* with white syndrome. The green cable tie marked the original position of the band when it was first observed and was used to measure disease progression. Photo: L. Raymundo

In subsequent visits, the distance from the nail to the new disease front is measured with a ruler. In many cases, especially in massive corals, you need a minimum of two nails per colony for common diseases that advance in a linear or annular manner as the distance from one nail to the disease front will vary depending on where you take the measurement. For branching colonies, a colored cable tie can be used to mark the original position of the disease front (again, place the cable tie at the edge of dead skeleton, not on live tissue) and the distance from the cable tie to the new disease front can be measured in future surveys (**Figure 4.7**). Average linear advance rate can be calculated per week or month as follows:

$$\text{Linear progression rate} = \frac{\text{distance from nail/cable tie to new disease front}}{\text{length of time of census (days/weeks/months)}}$$

Individual colony time-series photographs, those taken at the same angle and distance during successive monitoring visits, are powerful visual tools for examining the rate of advance of the disease front and host colony fate (Figure 4.8). These can greatly reduce bottom time, providing exactly the same position is used each time. However, you do need to factor in the time needed to process each photo in the lab. Photographs can be analyzed using image analysis software, which will allow accurate measurements to be taken of lesion size dynamics (increase/decrease) over time. Software packages provide instructions on how photographs should be taken. The National Institutes of Health (NIH) provides a free image analysis software package, ImageJ, which can be downloaded from their website: www.nih.gov. Another suggested package is CPCe, produced by Nova Southeastern University in Florida, which also provides a method for assessing cover using a series of random points. It can be found on their website: www.nova.edu/ncri/research/a10.html

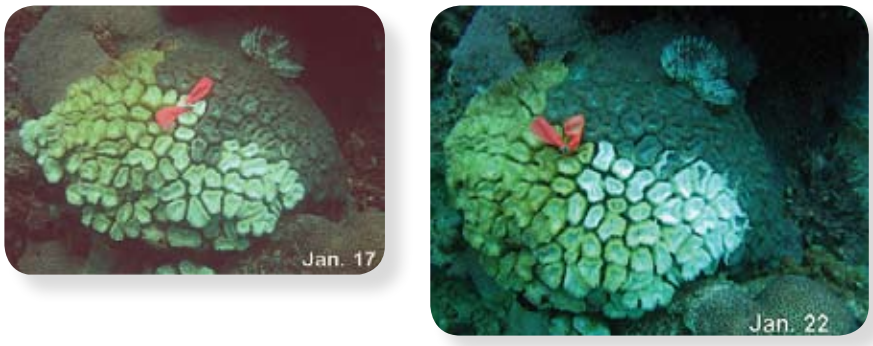


Figure 4.8 Five-day progression of a white syndrome infection on a tagged colony of *Lobophyllia*. Flagging tape tied to a nail provided both a visual reference and a scale bar to use a image analysis of the rate of tissue loss. Photo: K.Rosell

4.7 Studying links with environmental drivers

In Chapter 1, we discussed environmental degradation on global, regional and local scales and its links (potential and realized) with stress to coral communities. Because of this link, the importance of monitoring water quality concurrently with benthic monitoring has been recognized. Suggestions for how to do this are outlined below. However, logistical and cost issues may preclude a comprehensive plan.

Included in this section are guidelines for measuring some of the basic environmental variables which may have potential impacts on disease dynamics. Managers are encouraged to find out what water quality parameters are regularly monitored by other agencies in their area, and to develop collaborative arrangements so such data can be shared. Correlations between changes in environmental parameters and disease prevalence or incidence, or in the severity or rate of disease progression, can be tested with simple statistical tools, and by graphing a certain disease parameter against the environmental parameter in question (**Figure 4.9**).

Sea water temperature

At present, the only regional/global environmental variable of concern that can be easily monitored is sea water temperature, but it is an important one for diseases as well as bleaching events. An efficient way of monitoring sea water temperature is by means of submersible continuous recorders such as Tidbits (HOBO®) water temperature loggers. These loggers can be preprogrammed to record data at fixed intervals for a prolonged period of time. Earlier models require calibration before and after use with a clinical thermometer, as they can be inaccurate by up to 3°C. However, new versions (U22-001 units) can be deployed deeper (to 30m) and programmed to record data for up to five years. Data can be downloaded and re-launched underwater with a shuttle unit, and so can be immediately redeployed.

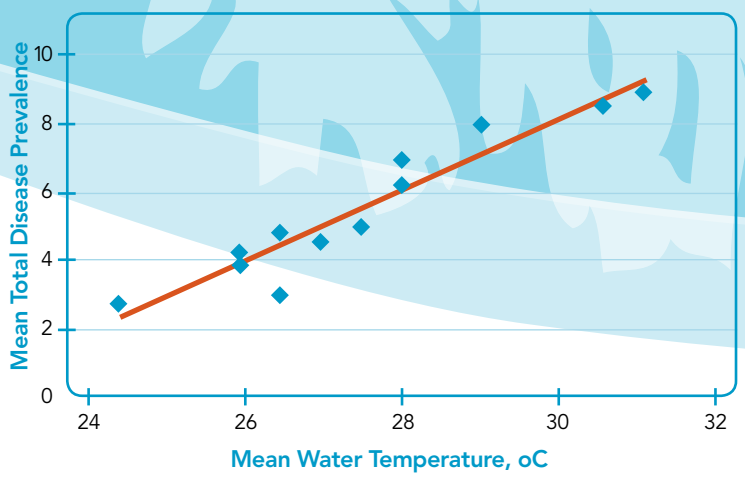


Figure 4.9 Hypothetical data set showing a strong relationship between increasing sea temperatures and total disease prevalence. Simple graphs, such as this one, can illustrate the strength of a relationship between a measured environmental parameter and disease.

Two units per sampling site are recommended; one deployed at shallow and one at deep depth limits of the monitored habitat or zone. If a strong environmental gradient exists among sampling sites, more may be needed. Temperature data can be summarized and plotted against disease prevalence data so evidence of co-variation between disease prevalence and seasonal changes of specific diseases or disease outbreaks can be detected.

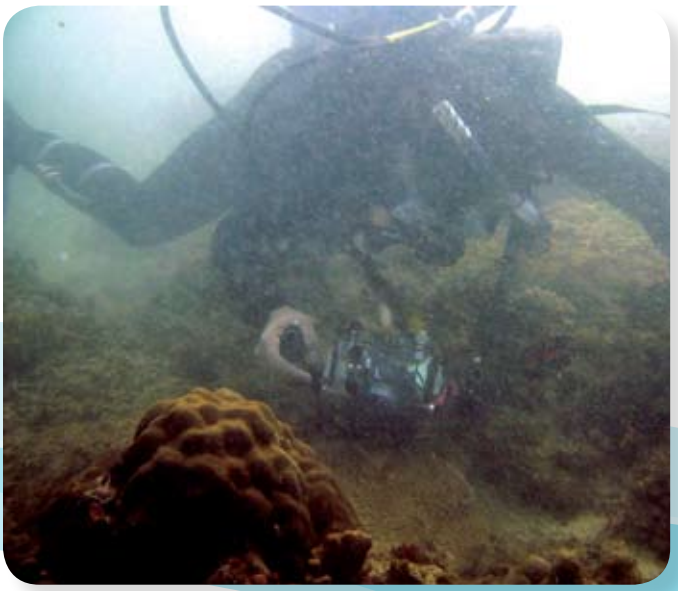


Figure 4.10 Chronic highly turbid conditions from silt deposition and resuspension can stress corals and other nearshore benthic organisms. Photo: L. Raymundo

Silt and sedimentation

Sedimentation is problematic in many coastal regions of the world where land use practices are poorly regulated and terrigenous soil and silt are deposited into nearshore communities via rivers and runoff (**Figure 4.10**). The sedimentation rate reflects the processes of deposition/delivery and resuspension of silt. It can be measured by setting replicate sediment traps in sampling areas and collecting and replacing the traps on a weekly or monthly basis (**Figure 4.11**). Sediments are processed in the lab following standard protocols (rinsing, air- or oven-drying, weighing) and sedimentation rates per unit time (day/week/month) are estimated. Sediment composition (i.e. the proportion of calcium carbonate to terrigenous materials) and granule size composition can also be determined. Such analyses can provide useful information regarding the sources of silt, its long-term impacts to benthic community health and structure, seasonality variations in silt delivery, and covariation with disease prevalence and bleaching events.

Figure 4.11 A sediment trap can be a deployed to quantify the amount of silt and other particulate matter settling onto a reef. Photo: E.Weil

If sediment traps and laboratory equipment for sediment analysis are unavailable, a less sophisticated method of estimating water clarity can be used. A Secchi disc provides qualitative estimations of clarity/turbidity, though it is not useful in shallow, clear water. A Secchi disc is a 30-cm diameter fiberglass, metal or wooden disc, painted white (**Figure 4.12**). (Note that those used for freshwater lakes are usually painted in black and white alternating quadrants.) It is lowered over the side of a boat attached to a weighted rope, and sunk until it is no longer visible. The length of rope deployed is then measured, the disc is pulled up until it is visible again and then lowered again until it disappears. The water depth at which the disc disappeared is used as an estimate of the depth of light attenuation. These measurements are rather qualitative and are therefore only used relative to other such measurements (i.e. when irradiance, cloud and wave conditions are similar). Nonetheless, they can provide an easy and inexpensive way to compare variability of water turbidity between sites and seasons. To standardize Secchi disc readings, always have the same person take the measurements, lower the disc on the shaded side of the boat, and always take measurements on sunny days, between 10am and 2pm, and at the same position at each site.

Other water quality parameters

Additional water quality parameters that may indicate environmental stress on corals include pH, nutrient load, and bacterial load. These, however, are more difficult to measure continuously if there are no funds for instrumentation and laboratory analyses. Managers are encouraged to link with laboratories, local environmental regulatory agencies, and even hospital laboratories, if it is perceived that any of these factors may be an important management issue that requires attention and monitoring.

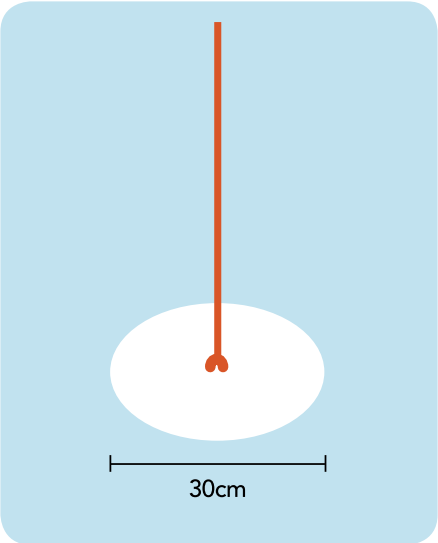


Figure 4.12 A Secchi disc is a useful and inexpensive tool for qualitatively comparing water turbidity between sites, providing it is used in a standardized manner.

Rainfall and river discharge

It has been verified that two diseases in the Caribbean, aspergillosis and white patch disease, are caused by ubiquitous terrestrial opportunistic pathogens (a soil fungus and a human intestinal bacterium, respectively) (52,65). While a definitive source of these pathogens has not been identified, it is a strong possibility that human activities were instrumental in either delivering these pathogens to nearshore reef environments or increasing their concentration in these environments.

In addition to being a source of potential pathogens in coastal waters, river discharge and non-point runoff are the main sources of terrestrial-based anthropogenic stressors such as fertilizers, pesticides, silt, sewage, and heavy metals from roads. Rainfall varies seasonally and alters river discharge, affecting salinity and pollutant loads in coastal receiving waters. Therefore, data on rainfall patterns may provide a proxy for seasonal shifts in the delivery of fresh water and pollutants onto reef communities. Often, rainfall is monitored by a local weather bureau which may also monitor river discharge. Such data are usually easy accessed, and may reveal links with seasonal changes and the prevalence of specific diseases. Where covariation is observed, further investigation is warranted to determine what stressors or pathogens may be linked with the disease impacts.

Box 4.1

Relocating difficult sites

We will address one difficult, but not uncommon, case here: setting up a sampling area which meets all criteria of the survey plan, and then relocating it in future survey trips when there are no convenient external spatial references (coastal reference marking, visible mooring buoys) or when conditions are difficult (strong winds, deep sampling sites, limited underwater visibility).

Before leaving the dock, tag all re-bars (or long masonry nails) and organize them in groups for marking each transect. When the reef site has been selected after preliminary survey dives, its waypoint (i.e. geographic coordinates) should be recorded in the GPS unit and in a field notebook, along with all reference information to the site (bearing direction from the dock, distance from shore, any shore positioning markings, etc.). After all transects have been established, construct a map of the survey area with the relative location of transects and any major bottom features that can help relocate the sampling area (Figure 4.13). Bearings should be taken with a high precision UW compass, and distances between markers estimated to help divers find the transects in subsequent trips.

Placing a mooring buoy to mark the sampling site is the quickest way to relocate the site and ensure anchor damage is prevented. But when this is not possible, a sub-surface buoy can be used. To avoid problems of limited visibility or to compensate for a less-than-accurate GPS re-positioning, another sampling site waypoint can be obtained to mark the position of a very distinct and large bottom feature within the sampling area. This feature should be clearly identifiable from some distance and will be the bottom spatial reference point, marked in the sampling area map and photographed for future use.

Obviously, the most practical way to relocate a site is with a GPS unit (Figure 4.14). However, the usefulness of this approach is dependent on the familiarity of the user with its limitations. Care should be taken to obtain the correct first GPS position, making sure that there are no drifting effects. To avoid this, mark the waypoint with a small anchored buoy with very little rope slack. Relocating the waypoint also requires counteracting any drifting effects, so use the same principle when returning to the site.

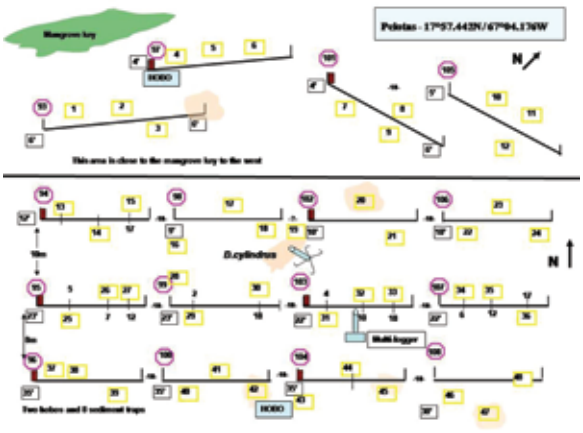


Figure 4.13 A map of transect locations at Las Pelotas in Puerto Rico. Additional underwater structures and data loggers were included for reference, to assist in relocating transects during subsequent visits.

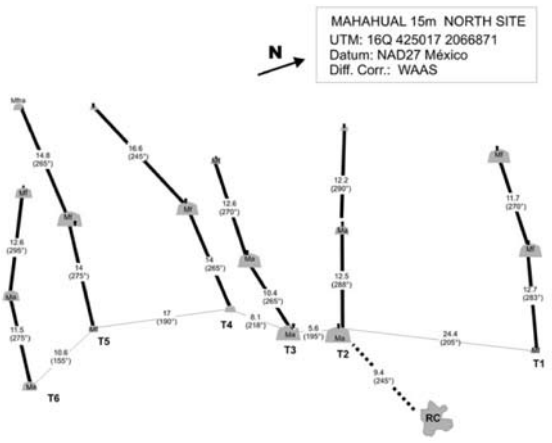


Figure 4.14 A map of a series of transects on the Yucatan Peninsula, Mexico. Transects were located by GPS for ease in relocation.

Chapter 5
Detecting and Assessing Outbreaks

In this chapter you will find:

How to define a disease outbreak in a coral community.

An overview of early warning signs of coral disease outbreaks.

Approaches for determining the extent and impact of a disease outbreak.

Detecting and Assessing Outbreaks

C.D. Harvell, C. Woodley, L. Raymundo and Y. Sato

5.1 When is a situation an "outbreak"?

An infectious disease outbreak is defined as a situation whereby the rate at which new hosts become infected increases. In other words, it is an unexpected increase in disease or mortality where it does not normally occur, or is at a frequency greater than previously observed. In **Figure 5.1**, we provide an example of outbreak dynamics for human diseases. As demonstrated in this graph, once the infected host population reaches a critical size, the number of new cases increases exponentially. As more individuals within a population are exposed to the disease, the number of new cases eventually declines, either through host recovery and immunity or death. The disease then becomes a long-term aspect of the population dynamics, becoming **endemic** and either reaching equilibrium with few occasional cases, or undergoing periodic outbreaks. **Figure 5.2** shows an actual outbreak of Bubonic Plague in Sydney in 1903. The epidemic followed this general pattern, becoming endemic afterwards until it was eradicated.

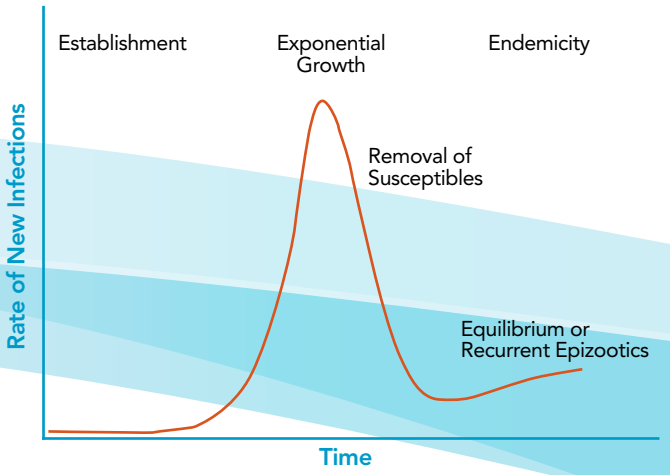


Figure 5.1 General example of dynamics of an infectious disease outbreak in a human population. An outbreak begins with random events that infect a small number of hosts. New cases occur exponentially after the infected population reaches a critical number. The outbreak dies out when the susceptible population declines either through death or increased immunity. Redrawn from Anderson et al. (4).

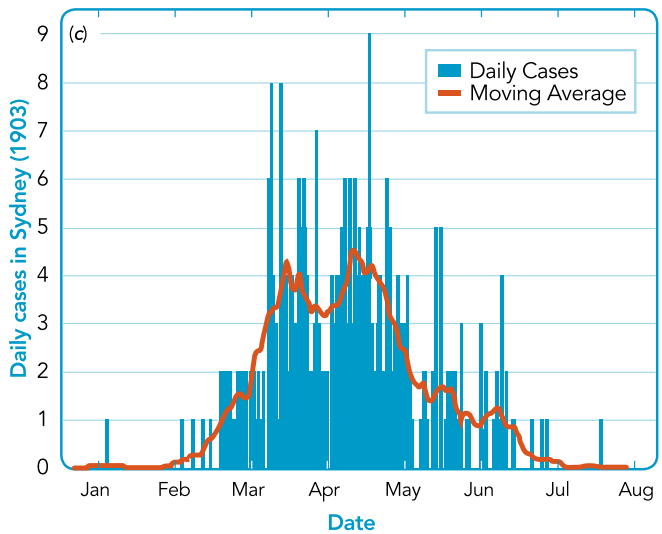


Figure 5.2 Disease dynamics of Bubonic Plague in Sydney, Australia, 1903. Modified from Keeling & Gilligan (3).

For coral, the situation is different; corals are a highly diverse assemblage of immobile, colonial animals that can only come into contact with other such animals via growth. The surrounding water in which they grow provides a means of contact with potential vectors of disease such as fish. In addition, portions of a colony may die in response to disease, while other parts may remain disease-free, providing an avenue for recovery or eventual re-infection. Therefore, we need to modify approaches applied to humans and other vertebrates to manage coral diseases. For instance, in corals, we may also apply the concept of an outbreak to a disease that suddenly affects a species previously thought to be resistant, or when there are signs of disease which do not correspond with any described in the literature (i.e. a potentially new or emerging disease). To develop a table similar to that of **Table 5.1** for a coral disease would require catching the outbreak in its early stage, marking all diseased

colonies in a defined area of reef, and monitoring the accumulation of new cases through time. This brings to the forefront the importance of long-term monitoring and regular random assessments, as it is during such activities that the early stages of outbreaks may be noticed. If a disease outbreak is observed early, then as much information as possible can be gleaned from the event, and a number of management options can be undertaken. Refer to **Chapter 6** for detailed information on strategies and options for managing coral disease.

Technically, an outbreak is defined as an R_0 value greater than one (4). R_0 is the "average number of secondary infections produced when one infected individual is introduced into a population of susceptible hosts". An estimation of R_0 can be calculated if enough information about disease transmission is known: (the number of contacts per unit time) x (transmission probability per contact) x (duration of infectiousness). A more direct approach of estimating R_0 from field data as the normalized accumulation of new cases (i.e. newly infected colonies) over time may be more attainable. Because corals are sessile, it is more possible to directly estimate R_0 in field populations than for most animals.

$$R_0 = (NT_2 - NT_1) / NT_1$$

Where

NT1 = the number of cases at time T1

NT2 = the number of cases at time T2

A disease will increase in a population with $R_0 > 1$; i.e. a diseased individual will more than replace itself. A disease will decline with $R_0 < 1$, and is considered endemic when $R_0 = 1$.

Table 5.1 presents some established R_0 values for certain wildlife diseases.

Table 5.1 Examples for estimation of the basic reproductive rate (R_0) for various pathogens in wildlife species (modified from Real and Biek 74).

Pathogen	Host species	Scientific name	R_0
Rabies virus	Spotted hyena	*Crocuta crocuta*	1.9
Phocine distemper virus	Harbor seal	*Phoca vitula*	2.8
Mycobacterium bovis	Feral ferret	*Mustela furo*	0.18–1.20
Mycobacterium bovis	Eurasian badger	*Meles meles*	1.2
Classical swine fever virus	Wild boar	*Sus scrofa*	1.1–2.1

Both R_0 and incidence can be estimated from field populations of marked corals (see example of incidence calculation in Chapter 3). The following example is provided for Black Band Disease of *Montipora* near Orpheus Island, Australia. Three 10mx10m quadrats were established in the vicinity, each encompassing 10-30 diseased colonies. Within the quadrats, all diseased colonies were marked. Quadrats were subsequently censused monthly for two years, and water temperature was recorded simultaneously. Monthly census intervals were considered sufficient as BBD does not spread very rapidly. In 2006 and 2007, outbreaks coincided with rising summer temperatures from November through to March, and declined in the cooler months of April through to August (**Figure 5.3**). R_0 was calculated as the average per capita increase in colonies with BBD (**Table 5.2**). An $R_0 < 1$ in February 2006 indicated that BBD cases were declining; an $R_0 > 1$ in December 2006 and January 2007 showed an increase in disease within the susceptible population. The extremely high R_0 value of 7.5 in December 2006 indicated the outbreak could become epidemic if temperatures remained high. However, by the following month, temperatures had begun to cool and a corresponding decline in the number of new cases was observed.

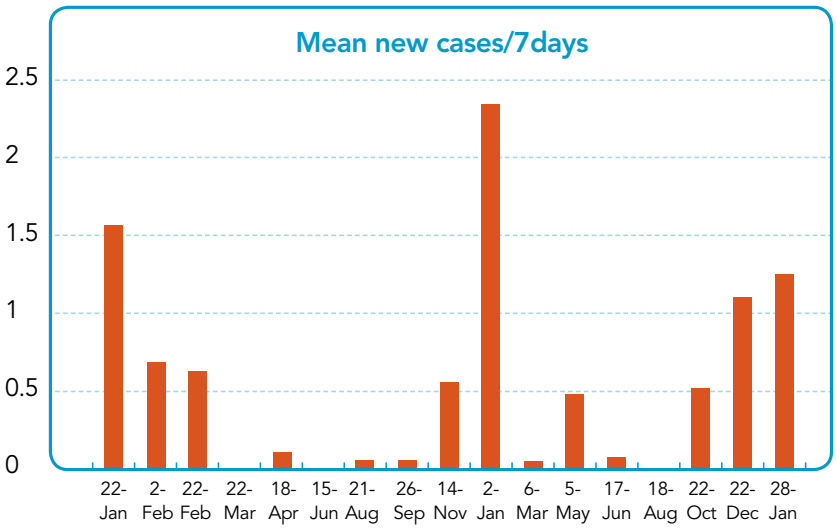


Figure 5.3 The mean number of new cases of black band disease (incidence) affecting *Montipora* species on the Great Barrier Reef, Australia. Data are shown only for months when sampling took place. Values taken from three quadrats monitored over a two-year period. This pattern closely follows a seasonal temperature trend and also illustrates the disease dynamics typical of an outbreak. Data printed with permission from Sato et al. (2).

Table 5.2 Estimations of R_0 from the change in the number of cases observed during monthly censuses of a black band disease outbreak on the Great Barrier Reef. Mean recovery, change in case number and new cases were calculated as the difference between the sampling months presented (i.e. Jan-Feb, Oct-Dec, Dec-Jan). Mean change in case number reflects both recovery and new infections. Mean calculated from three 100m^2 quadrats. Data printed with permission from Sato et al. (2).

Census month	Jan 06	Feb 06	Oct 06	Dec 06	Jan 07
Mean # BBD cases seen	14.00	16.33	0.67	5.67	15.67
Mean recovery		0.67		0.00	1.67
Mean change in # BBD cases	2.33		5.00	10.00	
Mean new cases seen		3.00		5.00	11.67
R_0		0.21		7.50	2.06

Outbreaks are usually short-lived, and should be treated with some urgency so as much information as possible can be collected while it is available. *Chronic diseases* can have equally devastating effects on populations and communities, particularly due to their potential effect on fitness. Yet because they are less strikingly visible, it is often more difficult to garner support for an investigation. Nevertheless, it is important to develop sound protocols for investigating and monitoring both transient outbreaks and chronic low-level disease, as both will factor as mechanisms of community change and indications of impacts to reef health.

5.2 Early warning systems for coral disease epizootics

Diagnostic tools for coral disease outbreaks lag behind those developed for bleaching events (For detailed guidelines see Marshall and Schuttenberg 75). Though global efforts to fill the knowledge gaps have resulted in great progress, we do not yet have the ability to accurately detect pending disease outbreaks before they happen. However, there are predictive tools available which are currently being tested for applicability to disease outbreak investigations. For example, regular monitoring of water quality parameters and environmental factors, as discussed in Chapter 4, can indicate potential stressful conditions for corals. Increased stress can affect resistance and increase susceptibility to existing pathogens. Over time, we expect to discern links between a change in a specific parameter and a change in the prevalence of one or more diseases. Therefore, monitoring environmental parameters may help to develop an early warning system for certain diseases.

The complex interactions among stressors, and their effects on corals and reef ecosystems, are generally poorly understood, making it difficult to assign a specific cause to local or regional declines in coral health. Persistent high levels of stress can cause sudden whole-colony mortality and disease may or may not be implicated as a direct cause of death. In contrast, chronic low levels of stress are likely to result in non-acute, sub-lethal effects on corals, which can have measurable (but not necessarily visible) effects on growth, reproduction or survival. Furthermore, chronic stress can reduce a coral's ability to resist disease, making a community of stressed corals vulnerable to an outbreak. Identification and mitigation of these stressors (i.e. toxicants, pollutants, sediment, nutrients, temperature) at an early stage of detection may enable the prevention of a disease outbreak.

Compromised health may manifest itself through shifts in coral physiology which may not be visibly detectable when surveying corals in the field. In the absence of acute mortality, biological indictors – or biomarkers – are being developed and tested to identify delayed or sublethal effects of exposure to stressors in coral. Biomarkers are substances that can be detected, sampled and measured to help characterize specific changes in health or physiological state. Antibodies, which can be sampled from vertebrate blood, are examples of biomarkers that can be used to detect exposure to a particular pathogen. Coral biomarkers can potentially detect cellular physiological changes in a coral before the coral develops visible signs of ill health such as tissue loss or partial mortality. This developing science is referred to as cellular diagnostics (70). Biomarkers from coral are being sought that can:

1. indicate exposure to a stressor or pathogen;
2. pinpoint an effect of stress or disease on coral physiology; or
3. show susceptibility to the stressor or pathogen.

As with human diagnostic assays, information about particular cell functions can be used to diagnose various disease states as a result of exposure to a specific stressor(s), and provide an indication of overall health in the coral. As this technology improves, it is hoped that simple, "user-friendly" diagnostic tools can be developed which test for levels of stress in corals and which might suggest increased susceptibility to disease before an outbreak occurs.

5.3 Techniques to quantify the extent and impact of an outbreak

When almost nothing is known about a disease (as is the case for most coral diseases) or at the time when a new or emergent disease is discovered, outbreak investigations are vital. An organized, systematic approach helps determine the extent and impact of the event, the causative agent(s), each agent's reservoir or source, and transmission routes between hosts. It can also identify knowledge gaps, help formulate hypotheses for further study, focus research goals, and help identify control or management strategies. When the cause of an outbreak is known (i.e. when we have identified the pathogen causing the disease which is rapidly increasing in a population), but the source (reservoir) or route of transmission remains unknown, much investigative work is still required (as was the case with *Vibrio shiloi*, the causative agent of one type of bacterial bleaching). In this case, investigations can focus on filling the knowledge gaps with regard to the ecology of the disease to better guide future control and management efforts.

Managers often have the most accurate and up-to-date information critical for an outbreak investigation. Their knowledge and historical information on local reefs are invaluable when it comes to informing the investigative process and determining the impact of a disease outbreak. The first objective in this situation is to create a "definition" of the disease. This includes identifying features that distinguish this particular disease from others and provides a route to understanding how and why the outbreak is occurring (76). The questions a manager can begin asking are:

- *'who'* is affected? (What species?);
- *'where'* is the outbreak located? (Including descriptions of surrounding environmental factors and investigating other possible outbreak sites);
- *'when'* is the event occurring? (Seasonal trends, the rate of movement through a population and rate of lesion progression on individual colonies);
- *'what'* features do the clinical and pathology analyses provide toward identifying a causative agent? (Protocol for this process is described in Chapter 3); and
- *'why'* or *'how'* did this occur? (This is the pathogenesis but can also include environmental factors which might have triggered the event, such as a sudden change in temperature or rainfall, or a toxin spill).

The initial information provided by the manager is critical to developing the **case history** and any subsequent investigation and laboratory work. Assistance in determining if an outbreak is occurring and how to respond to it can be obtained by contacting the Coral Disease and Health Consortium (CDHC) via email at cdhc.coral@noaa.gov, Dr. Andy Bruckner or Dr. Cheryl Woodley at NOAA (or see **Appendix 2** for a list of regional experts associated with the CRTR Coral Disease Working Group).

Managers who regularly monitor or assess disease will know what the characteristic prevalence levels are for a given area for each disease that has been documented and observed in that area. If a situation of concern is observed in the course of monitoring or rapid assessments, certain steps can be taken immediately. The most obvious situation is a much higher number of infected colonies than is normally observed for a given disease. Although we usually speak of an outbreak as referring to a single disease, this may not necessarily be the case. An outbreak in Palau in January 2005 involved multiple coral species and both white syndrome and black band disease, though the host range of the two diseases differed (35). An even more complicated case occurred during a Florida Keys 2003 outbreak, where a disease looked visibly identical in three acroporid host species. Extensive histological and molecular analyses, however, ruled them to be completely different pathologies (77). Below are a few simple steps that can be taken when documenting an outbreak:

1. Make an initial description of the affected site, which should include the following information: benthic composition (see **Chapter 4** for methods), depth range, reef zone/habitat, dominant benthic species, water clarity, current direction and relative strength, proximity to potential sources of stress such as river mouths, coastal construction zones, cities, and any additional information available that might shed light on why the outbreak is occurring at that place and time. Even if this information is qualitative, it can still be useful.

2. Identify and describe the characteristics of the lesions of the disease(s) you are observing, using the decision tree presented in Chapter 2. Photo-document lesions in all species affected. Make a presumptive field diagnosis of the suspected disease(s) you are dealing with; are you seeing a disease previously described and/or documented from the affected site? Or do the signs of disease you are observing not correspond with any previous description? This situation may represent a new or emerging disease in the area.

Figure 5.4. Top view of white syndrome outbreak spreading among at least 5 species in the genera *Lobophyllia*, *Mycedium*, *Merulina*, *Fungia*, *Favia* in Palau. Photo: B. Willis

Figure 5.5 Flagging tape tied to a dead portion of a colony or the substrate is an effective temporary means of marking an outbreak area boundary so that spread beyond an initial observation point can be tracked over time. Photo: K. Rosell

3. Develop a host range list – a list of all taxa apparently affected by each disease you are seeing (**Figure 5.4**). Make sure to note any colonies affected by more than one lesion type per colony. In addition, note all other taxa within the affected area which are not infected; resistance to a particular disease is equally important information. It is most helpful to identify to species wherever possible; at the very least, corals should be identified to genus.

4. Tag colonies for regular monitoring of disease progression. If possible, tag replicate colonies of each species affected for each disease observed. Attach tags to dead portions of the colony, or to nearby substrate, using flagging tape. Photograph all tagged colonies, being careful to place a scale bar or ruler in each picture. Photographs should attempt to include the entire lesion; depending on the size of the colony and the lesion, it may useful to take both a close-up picture, and a whole-colony picture (see **Appendix 3** for additional photos).

5. Using a systematic search swim pattern, (snorkeling or manta tows may be possible if the area is shallow), determine the physical boundary of the affected reef area. Mark the perimeter at regular intervals using flagging tape tied to corals or other underwater structures (**Figure 5.5**). Marking the boundary will allow you to determine the rate at which the affected area is spreading spatially in future visits to the site. Quantify this area by measuring maximum width and width perpendicular to maximum using a transect tape, and determine depth range.

6. Contact a laboratory with which you have a collaborative agreement, and make arrangements for sample analysis. Collect samples for microbial and histological analysis using the procedures outlined in Chapter 3. If you do not have a collaborative agreement with a laboratory, but feel this situation is urgent and requires resources beyond your capacity, contact the CDHC via email at cdhc.coral@noaa.gov.

7. Collect all environmental data available for this site, either from your own agency or others. This might include water temperature, rainfall, current wave height and tide patterns, sedimentation, and/or bacterial load.

8. Soon after you complete steps 1 to 5, perform rapid surveys (manta tows are particularly good for this) at increasing distances from the outbreak site, to look for sites of secondary outbreaks. Remember: do not go straight to "clean" sites from the outbreak site. Separate trips should be planned for these surveys, using equipment which has undergone the sterilization procedures outlined in Chapter 3. It is vital that those investigating a disease outbreak avoid spreading the disease to unaffected sites.

Figure 5.6 Remeasuring a black band disease front on *Montipora* sp. during a two year-long monitoring effort of a BBD outbreak on the Great Barrier Reef. The ruler in the photo provided a scale bar for both image analysis and measures of linear progression. Photo: Y. Sato.

9. Set up a monitoring schedule to revisit the principle outbreak site at regular intervals. If mortality is occurring rapidly, then it is advisable to resurvey at weekly or biweekly intervals, depending on your resources. When you revisit the site, photograph all tagged colonies, measure the linear progression of the disease front and note the health status of the colony (progressing, stasis, recovering, dead; **Figure 5.6**). Also look for new infections, either as additional lesions on previously-affected colonies or as new hosts. Over time, look for evidence of recovery, either from tissue resheeting over dead skeleton or through the recruitment of new colonies (**Figure 5.7**). Finally, at periodic intervals, repeat step 8, to make sure that secondary sites of infection are not developing.

And, finally, how is this information to be used? Data gleaned from a comprehensive documentation of an outbreak and subsequent monitoring is likely to include identification of susceptible and resistant species, species-specific mortality rates, species-specific recovery rates from tissue regrowth/resheeting, community recovery via recruitment, and responses of other reef biota such as macroalgae or sponges. All of these data can be used to examine changes in reef community structure as a consequence of the outbreak and coral mortality. **Chapter 6** addresses specific management options, where this information may be applied.

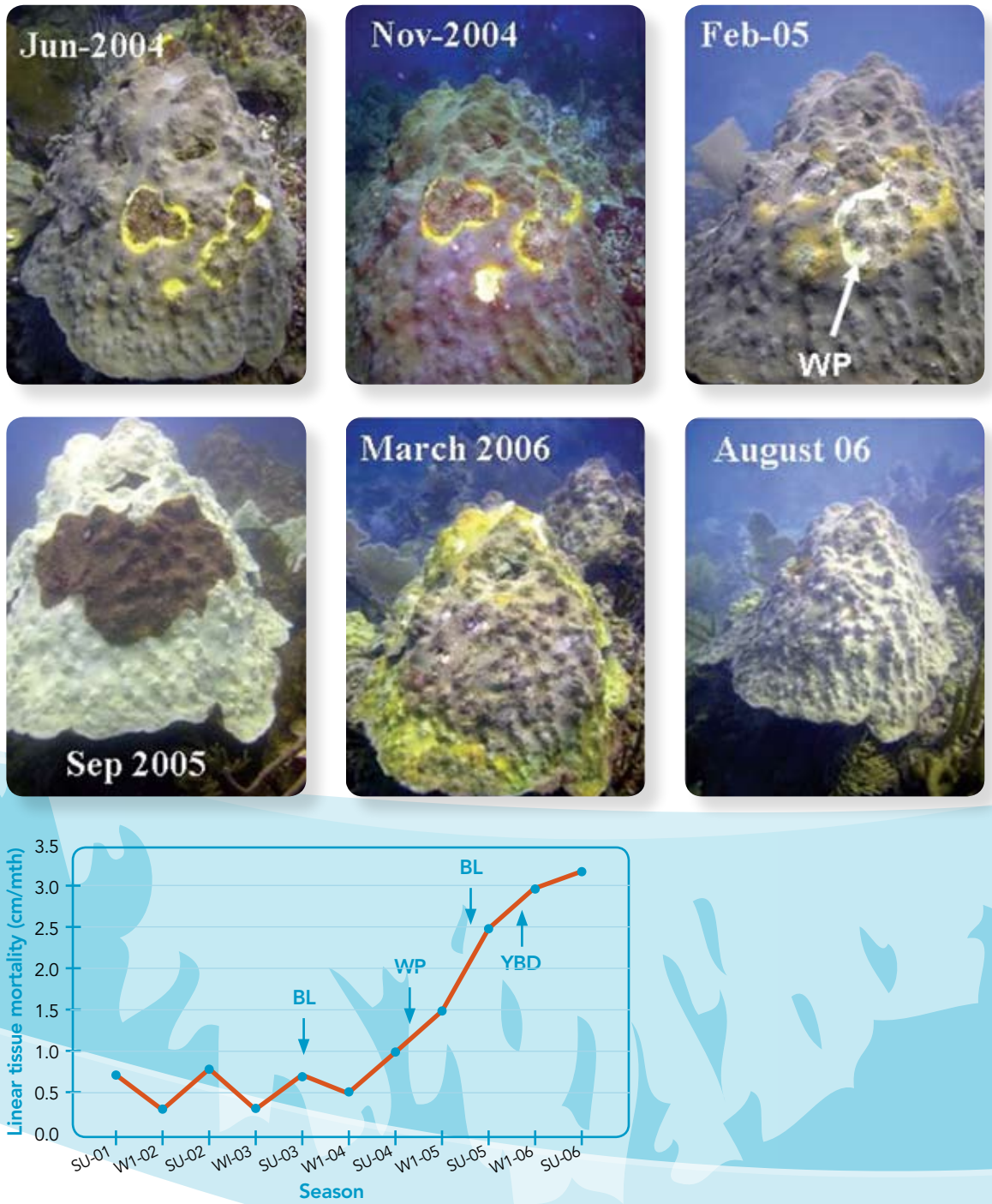


Figure 5.7 Time series photographs of one *Montastrea faveolata* colony with yellow band disease (YBD) in Puerto Rico monitored from 2004 to 2006. Several YBD lesions developed in June 2004, which expanded outwards during the follwing months. In February 2005, this colony also became infected with white plague (WP). In September 2005, all of the remaining living tissue bleached (see white area). After the bleaching event most of the colony became infected with YBD and by August 2006, the entire colony was dead. The graph shows the rate of this colony's tissue loss from summer 2001 to summer 2006, and the coinciding bleaching events (BL) and disease outbreaks. Photo: E. Weil

Chapter 6
Management Issues and Actions

In this chapter you will find:

A summary of the most current and comprehensive coral disease databases.

Where to go for further assistance and advice.

A look at management options for coral disease – general thoughts, what has been tried and current research efforts.

Management Issues and Actions

L. Raymundo, C. D. Harvell, A. Bruckner and C. Woodley

6.1 Management issues and challenges

Figure 6.1 Disease management for humans and wildlife involves quarantine, vaccination, culling, and education, most of which are not currently viable options for marine diseases. www.cdc.gov; www.dailygalaxy.com, www.rabies-vaccination.com; www.leolabs.com

The management of marine disease is a new frontier. Traditional management tools for human and wildlife disease control include quarantine, culling, vaccinating and education (**Figure 6.1**). However, only quarantine and education are currently viable options for dealing with coral diseases and examples where they have been applied are rare. We must consider certain fundamental differences between marine and terrestrial systems when looking at options for managing coral disease. Ocean water supports a rich and diverse microbial community. How many of those microbes are pathogenic, or potentially so, remains unknown. Because ocean water supports such a vast microbial community, marine systems are considerably more "open" and connected to each other than terrestrial systems. Additionally, our incomplete knowledge of corals, their diseases, their resistance capabilities and the influence of environmental stressors on their health, pose additional challenges to developing workable management options.

However, the impacts that diseases are having on coral populations highlight an urgent need to develop management options concurrently with scientific investigation of diseases. Although the science is still in its infancy, there are practical steps that reef managers can take to manage coral disease. The current perception that coral diseases are "unmanageable" is untrue and managers are in the unique position of being able to develop and test options for controlling disease spread. However, these tests should be conducted with careful planning: any potential management tool should be tested via a properly designed experiment and, most importantly, results should be reported. The authors of this manual are available as contact persons for advice on this (their contact details are listed in **Appendix 2**). Below we present some areas which could guide coral disease management.

6.2 Global disease databases: options for managing information

Resource management must be based on sound science. In turn, scientific information must be interpreted and communicated effectively so it facilitates meaningful management decisions. As a growing field of science and an urgent management issue, coral disease is the focus of a world-wide research effort which is generating an enormous amount of information. Due to the global implications of disease impacts and the pressing need to standardize all aspects of disease investigation, data generation and management are two very important features contributing to this effort.

There are a number of databases, accessible to the public via the internet, which contain various types of information on coral diseases. Such databases are integral to synthesizing information from diverse sources and rely heavily on the addition of accurate information as it becomes available. A repository for global information which is accessible, accurate, and helpful is key to developing and testing management options. Below is a brief description of the most current and comprehensive of these.

The Global Coral Disease Database

The Global Coral Disease Database (GCDD), available at http://www.unep-wcmc.org/GIS/coraldis/index.cfm, is the most comprehensive compilation of coral disease information. It was developed by the U.S. National Oceanic and Atmospheric Administration's (NOAA) Coral Reef Conservation Program in conjunction with the United Nations Environmental Program's (UNEP) World Conservation Monitoring Centre (WCMC). It is a web-accessible GIS database that compiles records of disease observations and tracks their spread over time, by geo-referencing disease locations and plotting their occurrences onto WCMC coral reef distribution maps. The GCDD includes an online mapping tool (a prototype IMAPS tool) that enables users to search and plot data by disease name, year, or country, with zoom capabilities and a full information sheet for each line of data. It also has the option to separate reports into novice and expert data. For each disease, information can be obtained regarding: its global and regional occurrence and abundance, affected locations (i.e. country, reef, latitude and longitude) and species, and any available site-specific data on prevalence, incidence, and extent of mortality, by querying the database or using the mapping tool.

The database is linked to WCMC's Protected Areas and Coral Species databases, and contains coral disease identification tools and a photographic key to western Atlantic diseases. All in situ observations on prevalence, host range, global geographic distribution, and mortality for coral diseases are compiled for the period 1972-2006 from peer-reviewed journal articles, technical reports, regional monitoring data from Atlantic and Gulf Rapid Reef Assessment surveys, CARICOMP surveys, Global Reef Check monitoring efforts, and reports submitted by researchers. These datasets reflect wider spatial coverage of disease surveys, repeat surveys, and increases in the types of diseases and species affected over the last few years. The GCDD currently contains over 8000 records of disease, and includes reports of over 40 coral diseases from the western Atlantic, 28 from the Indo-Pacific and five from the Red Sea. Contact Dr. Andy Bruckner, Andy.Bruckner@noaa.gov for more information, or to submit information on coral diseases.

6.3 Where to go for assistance and advice

It is important to be aware that there is a network of dedicated and qualified scientists and managers who can be contacted for assistance, information, and advice. In many remote locations, managers often juggle numerous projects and responsibilities with few resources, and may even be forced to take on responsibilities for which they have no formal training. One objective of this book is to provide assistance to individuals who may be working in relative isolation. Another objective is to expand the current network of individuals working in the field of coral disease and coral reef management, and to assist managers who could benefit from additional expert support in obtaining the advice and information they need. Further, we wish to convey the importance of communicating research and management experiences in the field of coral diseases, either through publication or presentation at symposia. There are many geographic locations for which there is no information currently available on the status of coral diseases; this is particularly true for much of the Indo-Pacific and East Africa. By linking managers and scientists with other managers and scientists, information can be exchanged and productive collaborations can be forged. Below, we present two organizations whose members can be contacted for information and can answer specific questions beyond the scope of this book.

The Coral Reef Targeted Research Program, Coral Disease Working Group

The Coral Disease Working Group (CDWG) as described in Chapter 1 (**Box 1.1**) is one of six working groups of the Global Environment Facility and World Bank's Coral Reef Targeted Research (CRTR) program, launched in 2005. The CDWG maintains collaborations in support of coral disease research at each of the CRTR's four regional Centers of Excellence: the Marine Science Institute/Bolinao Marine Laboratory of the University of the Philippines, Philippines; University of Dar Es Salaam, Institute of Marine Science, Zanzibar, Tanzania; University of Queensland Heron Island Research Station, Queensland, Australia; and Unidad Académica Puerto Morelos, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (National Autonomous University of Mexico, Institute of Marine Sciences and Limnology, Puerto Morelos, Mexico). All Centers of Excellence have the capability to conduct field assessments of local infectious coral syndromes and can provide

information on sample collection and where to send samples. The CRTR website can be found at www.gefcoral.org. This site describes current research efforts in coral reef ecology, and provides additional contact information. Contact information for individual members of the Coral Disease Working Group of the CRTR can be found in **Appendix 2**.

The Coral Disease and Health Consortium

The Coral Disease and Health Consortium (CDHC) was created in 2003 as a cooperative effort linking representatives from U.S. agencies involved in coral reef management. Partners include the U.S. NOAA, the U.S. Environmental Protection Agency (EPA), the U.S. Department of Interior (primarily, National Park Service and Geological Survey), U.S. Coral Reef Task Force agencies, not-for-profit organizations and academia, both national and international. Currently, the group is involved in health assessments; outbreak responses within the U.S. and associated territories; research and development for diagnostics and pathology; an International Registry for Coral Pathology; and capacity building efforts that include training, technology transfer, and strategic research planning. For a downloadable copy of the statement of purpose of the CDHC, refer to: www.coralreef.gov/library/pdf/FInal%20CDHC%20plan.pdf. You may contact the CDHC directly at this email address: cdhc.coral@noaa.gov, or either Dr. Andy Bruckner or Dr. Cheryl Woodley at NOAA. Please see **Appendix 2** for their contact information.

6.3 Management options in the face of incomplete knowledge

6.3.1. Some general thoughts and options

Management strategies for coral disease must develop as a result of careful and rigorous testing and reporting of results. As we stated earlier, the traditional methods of culling and vaccination for disease control currently have limited application for coral disease management. However, certain aspects of coral life history may lend themselves to disease control if they are incorporated into a management strategy: corals, unlike most other wildlife species of concern, are immobile; once a diseased colony has been located, it will remain in that location and can be counted and revisited (and potentially treated, if viable methods are developed). Furthermore, corals have the potential to regrow over dead skeleton by resheeting. In these ways, corals function more like plants. Keeping these characteristics in mind may help managers to "think outside the box" and develop management strategies unique for corals and other sessile, benthic organisms. Experiences from the agriculture and plant disease literature may provide guidance to management of coral diseases.

As mentioned previously, proving the cause of a disease is a difficult, lengthy process, beyond the scope of most managers and their laboratories or field stations. However, one does not need to know the causal agent of a disease to take management steps. A first important step is to develop a working knowledge of the diseases and compromised health states present in a given management area (i.e. to know what is normally present, and at what levels, in the coral community). It is only by understanding what represents 'baseline' conditions that one is able to assess what represents above-normal disease levels and their potential for increased mortality.

Building on this idea, long-term monitoring data sets should be viewed as valuable tools for local management agencies. Responses to natural phenomena such as seasonally warm sea surface temperatures or periodic corallivore outbreaks can be observed both in the long-term and in a larger geographic context to identify sites that show greater or lesser resilience to such impacts. It is now considered good policy to identify particularly vulnerable sites (as well as those that show high resilience and resistance) for increased protection and management (78). Such data sets can also be used to bring about policy change (such as guidelines for coastal development) and to assess the impacts of events such as ship groundings and chemical spills. In many jurisdictions, there may not be a legal framework in place to force the perpetrators of such events to remediate damage. A comprehensive data set which quantifies "before and after" impacts can be used as a basis for developing laws and regulations to guide remediation.

Figure 6.2 Intensive aquaculture, such as this fish pen, can deliver high concentrations of organic nutrients, antibiotics and pesticides to coastal ecosystems, with unknown impacts to health. Photo: L. Raymundo

This, then, is another important management tool: by controlling the input of anthropogenic stressors on reefs, we can optimize conditions favorable for reef health and coral growth. Ultimately, this might be the most powerful and successful management strategy, one with multiple positive consequences on all coastal ecosystems, and one whereby local management agencies can exert some control. Demonstrated links between coral disease and specific anthropogenic inputs can be used as political leverage to improve water quality, particularly in those local economies dependent on diving tourism and reef health. To date, it appears that many infectious syndromes of corals are caused by opportunistic bacteria, with many representatives of the genus *Vibrio*. *Vibrio* spp. are among the most common bacteria in the ocean (79), and members of this genus are also responsible for certain important water-borne human diseases such as cholera (*Vibrio cholerae*; 80). Similarly, the ciliate infections, such as brown band disease (BrB), skeletal eroding band (SEB) and Caribbean ciliate infection (CCI) are likely to be opportunistic. For these infections, the best management option is to control or reduce the stress in the environment, thereby improving the corals' chance of resisting or recovering from infections (**Figure 6.2**).

6.3.2. What has been tried?

There is evidence to suggest corals that survive a bleaching episode may later succumb to an opportunistic infection, as their resistance is lowered by the stress of bleaching (42,81). In such cases, imposing a quarantine on a reef acutely impacted by either bleaching or disease may be a viable option. The reef can be closed to human activity by prohibiting diving and snorkelling for a period of time. This was successfully undertaken in Florida in 2003 during a disease outbreak. A Florida Keys reef was closed to all human activity except approved research and investigation for 60 days (B. Causey, pers. comm. and Federal Registrar 82). This management approach has a number of potentially positive consequences. First, when dealing with an outbreak of an apparently unknown disease, the possible risk to humans is very real; closing access can prevent an impact on human health. Second, not only can divers break and abrade corals, they can also potentially transmit pathogens between reefs via contaminated gear. Many dive operations take large numbers of divers to several reefs in a single day, which could greatly facilitate the spread of infection between reefs via dive gear. Closing a reef can isolate an infection and limit its damage.

Figure 6.3 Applying putty to a yellow band disease front slowed the progress of disease. Photo: A. Bruckner

More direct management actions to alleviate infections may be possible in the case of a few pathogens. For example, there has been some success in controlling the spread of black band disease (BBD) during warming anomalies by aspirating the band using large syringes or pumps. Clay or underwater epoxy putty can then be placed directly over the band. These methods were first developed by Harold Hudson in 1986, and since then have been adapted by other scientists (Causey, pers. comm.). If clay is used, all cyanobacterial filaments must be removed; it does not persist long and, the filaments will eventually emerge from within the clay (83). Putty is harder to work with as it does not adhere as well. However, it is more permanent and effectively halts any cyanobacterial growth left in underlying coral skeleton after aspiration. This has also been successfully attempted with yellow band disease, white plague and white band disease. Preliminary results showed that band progression was slowed, and in more than 60 percent of cases, the disease was arrested (Bruckner, pers. comm.; **Figure 6.3**). If this approach is to be attempted, it should be done with great care to avoid spreading cyanobacteria and other microorganisms comprising a diseased band to surrounding corals.

Another practice that has been attempted is shading highly susceptible reefs during bleaching events. It necessitates deploying shade cloth that eliminates approximately 60 percent of incoming irradiance and requires 10 to 12 days to take effect. However, shading corals for this length of time may also exacerbate bleaching, so this practice is not recommended.

Finally, experiments have shown that black band disease can be eliminated and the rate of appearance of new infections can be reduced through re-introduction of herbivorous urchins *Diadema antillarum* into habitats where they were formally abundant (83). Through grazing behavior, these urchins reduce the potential for algal competition with corals, thereby reducing the likelihood of injuries that may facilitate an invasion by pathogens, and directly removing the substrate that cyanobacterial filaments require for attachment.

While antibiotics are successful in treating systemic infections of humans and wildlife, it is not recommended to apply antibiotics in open marine ecosystems, and especially to corals. The coral holobiont is an extremely complex consortium involving beneficial surface bacteria and indiscriminate use of antibiotics without proper understanding of this complexity may do more harm than good.

One final point: currently, the "treatment" of coral diseases in the traditional sense is not considered feasible for eliminating disease during an outbreak. It is costly and time consuming, and infections tend to be in varying states of progression at any given point in time. However it may be a viable approach to save certain high-value colonies, such as massive reef building corals, or rare species that are viewed as particularly important.

6.3.3 Current research efforts

One experimental program underway at the University of Tel Aviv involves phage therapy of corals. Bacteriophages are viruses that kill bacteria and many are extremely specific in the bacteria they kill. This program involves the isolation of specific phages that prey on bacteria pathogenic to corals. In test cases, the bacteria treated were *Vibrio coralliilyticus* and *Thalosomonas loyaeana*. Scientists were able to successfully isolate phages, introduce them to tanks with infected corals and increase the survival rate of the corals. However, transferring such a technology to a reef system has serious logistical and ethical issues, and to date, this has not been attempted.

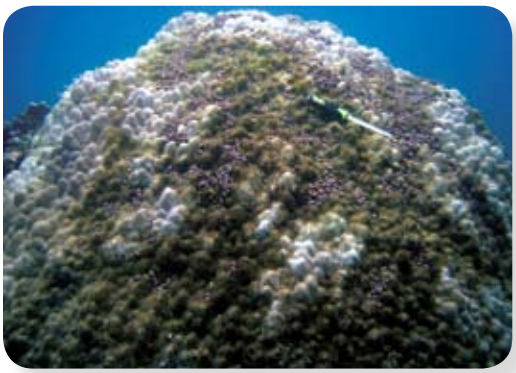


Figure 6.4 A population explosion of the gastropod corallivore *Drupella cornus*. This population killed a several hundred-year-old massive *Porites* colony, among many others, in the central Philippines. Note the white patches of recently-killed tissue and the brown patches covered with recruiting macroalgae. Little healthy tissue remains on this colony. Photo: L. Raymundo

Another potentially important area of research is the identification and removal of vector organisms. Vectors can transmit pathogens between hosts by contacting the hosts during predatory, commensal or competitive interactions. Recent research efforts have uncovered a number of links between host corals and organisms that transmit a pathogen. The marine

corallivorous fireworm, *Hermodice carunculata*, for example, is thought to transmit *Vibrio shiloi*, which causes bleaching (84). The green calcareous alga *Halimeda opuntia* can transmit the causal agent for white plague, *Aurantimonas coralicida*, to host corals it brushes against (85). The Caribbean gastropod *Coralliophila abbreviata* is a vector for a white syndrome that affects *Acropora* (86) and the three spot damselfish (*Stegastes planifrons*) has been shown to transmit black band disease between colonies via predation (87). However, we do not advocate the indiscriminate removal of all corallivores, as there is ample evidence from terrestrial systems of unpredictable consequences resulting from either the addition or removal of species to and from ecosystems. That said, there are certain species that cause an inordinate amount of destruction because their populations go through "boom and bust" cycles. If it was established that any of these highly destructive corallivores, such as the Crown-of-Thorns starfish (COTS), *Acanthaster planci*, or the gastropods *Drupella* spp. (**Figure 6.4**), transmitted a pathogen, then removal of these predators could potentially control the spread of disease. Indeed, in the case of COTS, removal efforts during outbreaks are regularly attempted. Caution must be exercised, however, as there may be other impacts to the ecosystem of such practices. For example, prior to a full understanding of COTS biology, it was a common practice for volunteer divers to cut the starfish into pieces. Due to the regenerative powers of starfish, this acted as an asexual means of reproduction and virtually created more starfish. When this practice was stopped, volunteers began eviscerating the starfish and leaving them on the reef. However, trauma to the gut initiates spawning in COTS, and so created the next generation of starfish. Today, it is understood that the most effective means of controlling COTS during an outbreak is simple removal from the reef.

Figure 6.5 Here, college students developed hands-on activities to teach grade school students about the importance of coral reefs and the organisms that depend on them. Photo: B. Baldwin.

Finally, one cannot underestimate the importance of education and public awareness efforts (**Figure 6.5**). Managers should make every effort to disseminate to the public locally-relevant information on coral diseases and their potential impacts. Managers may also focus their attention on target groups who interact regularly with the reef: fishers, recreational divers, and diving tourism operators and their clients. In places where diving tourism is a major source of revenue, there may be hundreds of visitors to a single reef each day. Poorly-trained dive instructors and tourist dive guides can wreak havoc on a reef by encouraging physical contact with corals and other benthic organisms (**Figure 6.6**). However, educating and involving such individuals in conservation efforts can have productive results. Many dive operators would like to know how they can adopt an eco-tourism approach to their operations, and many are enthusiastic about participating in monitoring activities. Harnessing such enthusiasm will provide managers with additional observers underwater, and the only efforts that are necessary are some initial training and regular communication.

6.4 Procedures and practices to promote better management

Details of some of these procedures have been outlined elsewhere in this book, but to apply them specifically in a management context, we summarize them here as well.

1. Restrict translocation of corals to prevent movement of disease.

   Corals are translocated locally, regionally, and globally for a variety of reasons (rehabilitation, aquaculture, and the aquarium trade, to name a few). Such practices should only be allowed if quarantine conditions are possible. No corals with visible signs of disease or compromised states should be collected and transported to other field locations, no matter what the purpose. Transport to quarantine facilities should be under strict biocontainment conditions that prevent release of diseased materials or water into the environment.

2. Provide guidance for proper handling and containment regimes during coral disease experiments.

   Chapter 3 of this book discussed such procedures in detail. Managers may be in a position to create regulations and enforce rules locally, and it is hoped that the information provided in this book will be used for this purpose. If additional guidance is necessary, please consult the list of experts in **Appendix 2**.

3. Monitor proposed coral management and research activities, as well as rehabilitation or remediation activities, to minimize or avoid ethical and legal problems with the potential spread of disease.

   Again, managers are in a unique position to understand the ethical and legal issues involved with the use and transport of coral, and the manipulation of coral reef communities. Ensuring the proper procedural controls on these activities will help manage disease.

4. Promote the use of universal precaution measures when dealing with diseases in the field.

   The suggestions outlined in Chapter 3 and 4, such as working from uninfected to infected areas, and sanitizing SCUBA gear and equipment when moving between reefs etc. can be worked into a formal regulation and permitting procedure, to ensure compliance.

Figure 6.6 Many dive operations run by poorly-trained instructors encourage their clients to touch and handle corals. The potential for abrasion and breakage, as well as disease spread, is very high with such practices. Photo: D. Burdick

5. Encourage ethical behavior and improved sanitary practices among divers and other users of the marine environment.

   As outlined above, disseminating this type of information to the public will educate recreational users in their role in this management process, and assist managers in promoting reef health.

6. Communicate and report disease outbreaks and interventions.

   Communication of disease events and efforts to manage them, even if such efforts were unsuccessful, is essential. Only through coordinated, collaborative effort will significant progress be made. Such information can be communicated in many ways: disseminating technical reports, publishing in the scientific literature, presenting talks or posters at scientific meetings, and submitting data to the GCDD are the most efficient means available and will reach the largest number of professionals.

6.5 A look to the future

This book is a first attempt to provide resource managers with a practical approach towards identifying, assessing, quantifying and monitoring coral disease. We hope that it provides useful and practical information and resolves some of the current issues relating to coral disease. However, we recognize that given the extraordinarily rapid rate of accumulation of information, certain areas of this book may become obsolete fairly quickly. It is our hope that future editions and other publications produced by this working group will provide updated information, as well as answers to some of the more urgent questions we are confronting now.

Given the current focus on research for management and with a concerted global effort to link managers and scientists, we predict that future efforts will result in a much greater understanding of coral disease. This understanding will undoubtedly have a ripple effect, resulting in further comprehension of related topics. So far, our initial efforts to understand coral disease have expanded our knowledge in the areas of coral histopathology, microbial ecology, the coral holobiont, epidemiology and veterinary medicine, to name a few related fields. In the future we expect managers will be equipped with improved histological and molecular diagnostic tools to identify causative agents. Improved understanding of the genetic diversity of coral communities, and their resilience to absorb changes to their environment may increasingly be used as criteria for selecting reefs in urgent need of protection (i.e. Marine Protected Areas, sanctuaries, reserves).

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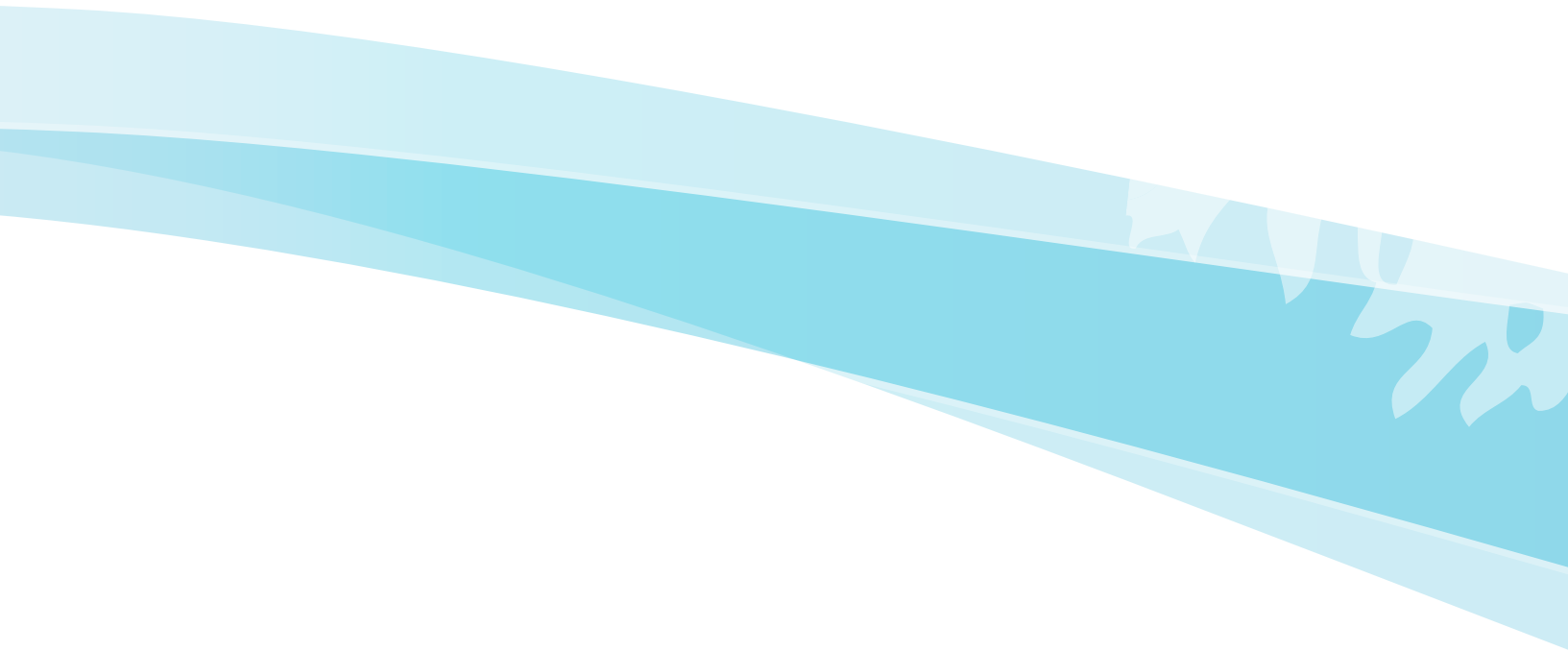
Acknowledgements



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Appendices

In the appendices you will find:

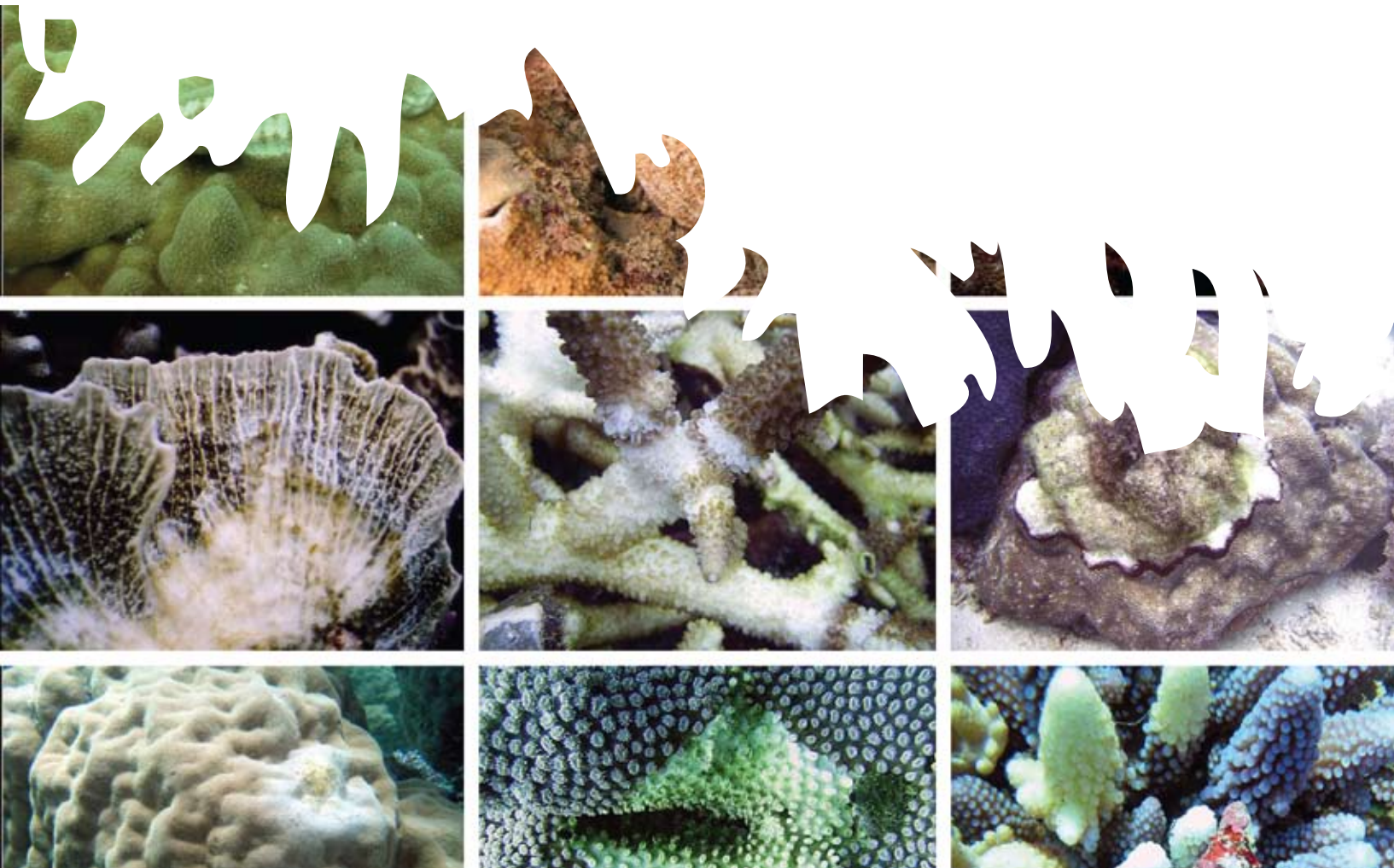
Glossary and acronyms.

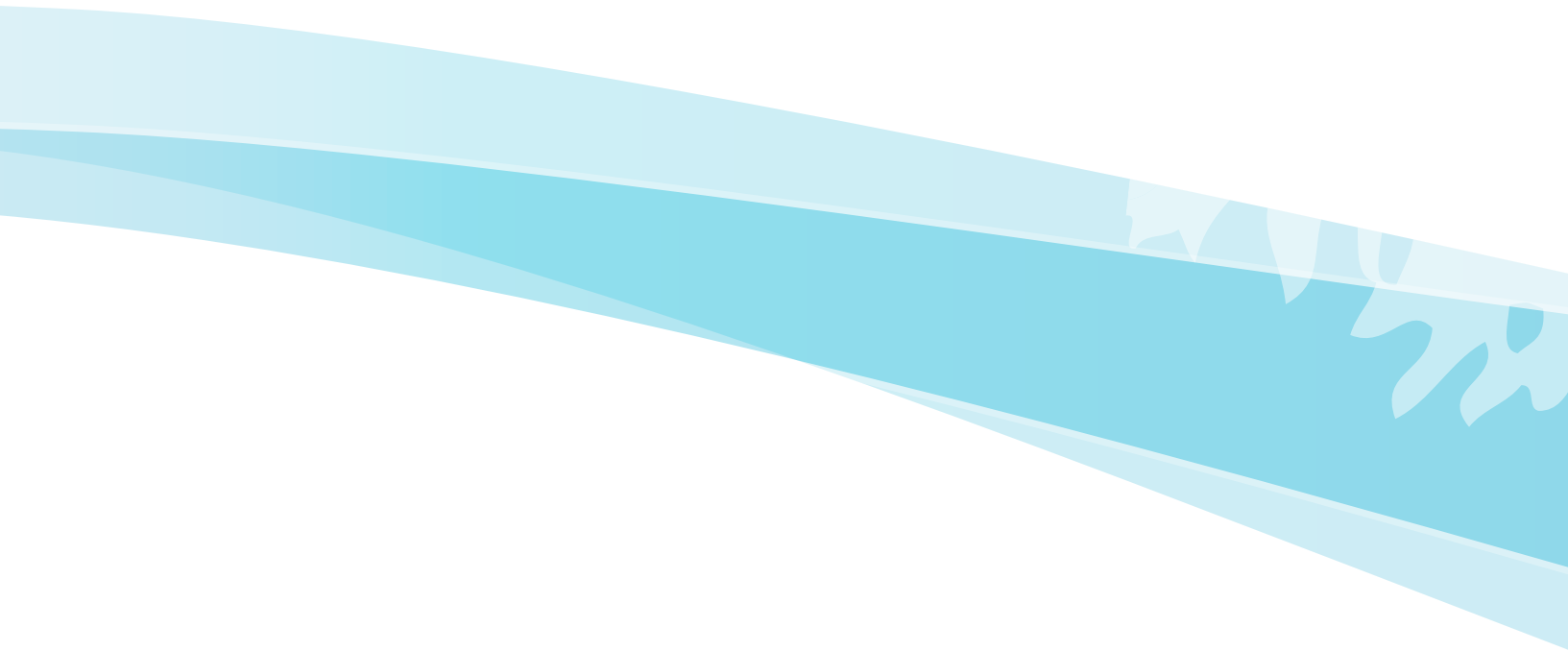
Regional contact list of coral disease experts.

Supplementary disease and compromised health photographs.

Data sheets currently used for assessment and monitoring.

Supplementary disease descriptions.





Appendix 1:

Glossary and acronyms

Glossary

(Terms defined in the glossary are bold in the text)

Acute disease – A disease that has a relatively rapid onset (i.e. influenza or food borne-diarrhea; 88).

Agent – A factor capable of producing an effect (i.e. a cause of disease; 88).

Biomarker – a substance that indicates the status or condition of a specific biological property (70).

Case history – A chronological record of significant events and observations made during a disease investigation.

Chronic disease (infection) – A disease or infection that has a relatively slow onset (i.e. cancer; 88).

Coenosteum – Skeleton deposited outside and between the corallite walls of the polyps of a colonial scleractinian (71).

Corallite – The calcium carbonate skeleton deposited by and around a single polyp (89).

Corallivore – An animal that eats live coral tissue; certain parrot fish, gastropods such as *Drupella* and *Cyphoma,* fireworms, and the starfish *Acanthaster planci (90).*

Diagnosis – The determination of the nature of a disease (88).

Disease – Any impairment that interferes with or modifies the performance of normal function, including responses to environmental factors such as nutrition, toxicants, and climate; or infectious agents, congenital defects, or combinations of these factors (91).

Endemic – Present in a susceptible community at all times, but in low frequency (92).

Environment – An area where agent and host interact to produce disease (93).

Emergent disease – Any resurging disease that was previously at low levels in a population, or a new disease in a population (94).

Epidemiology – The study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems. For non-human animals, the term **epizootiology** is used (92).

Epizootic – Occurrence of disease at levels above what is expected in a population (88). Applies to non-human animals.

Histology – The study of tissues and cells on the microscopic level (95).

Holobiont – The animal-plant complex formed by interactions between a coral polyp, its endosymbiotic algae (zooxanthellae), and its associated bacterial community (44).

Host – An organism that harbors the agent causative of disease (93).

Host range – The collection of species which are susceptible to a given pathogen.

Immunity – Non-susceptibility to infectious or toxic agents (96).

Incidence – The number of new cases of disease over a specified time period in a population at risk for developing the disease (92).

Infectious – Capable of causing infection (88).

Lesion – Morphologic changes that accompany disease; manifestation of disease (88).

Necrosis – Cell death characterized by irreversible damage, the earliest of which occurs in mitochondria (modified from Stedman 88).

Octocoral – Corals with polyp tentacles and mesenteries in multiples of 8; includes soft corals, sea fans, *Heliopora*, and sea pens (89).

Opportunistic infection – Infection caused by existing microorganisms not normally pathogenic (88). For instance, this may occur if environmental conditions change, thereby stressing the host and increasing its susceptibility to the microorganism.

Outbreak (see **epizootic**)

Pathogen – Any disease-producing agent (96).

Prevalence – The number of diseased colonies relative to the total number of colonies present within a defined area of survey at a given point in time. Usually expressed as a percent: (no. disease cases/total no. colonies) *100 (modified from Gordis 92).

Progression – Increasing in severity (96).

R_0 – "R naught"; the average number of secondary cases generated by one primary case in a susceptible population (4).

Reservoir – An alternate host or passive carrier of a disease-causing organism (96).

Resistance (see Immunity)

Scleractinian – Polyps with mesenteries and tentacles in multiples of 6; true stony corals; Acroporidae, Poritidae, Pocilloporidae, etc. (89).

Severity – The percent of a colony affected by a disease (97).

Sign – Any objective evidence of a disease perceptible to an observer (96).

Stress – The sum of biological reactions to an adverse stimulus that disturbs an organism's homeostasis (96).

Syndrome – A **set** of **signs** or a **series** of events occurring together that often **point** to a **single disease** or **condition** as the **cause** (Dept. of Oncology, University of Newcastle Upon Tyne).

Transmission – A passage or transfer of a disease from one individual to another (96).

Vector – An animal that transfers an infectious agent from one host to another (96).

Virulence – The relative pathogenicity of a microorganism (95); how easily it causes damage to host tissue.

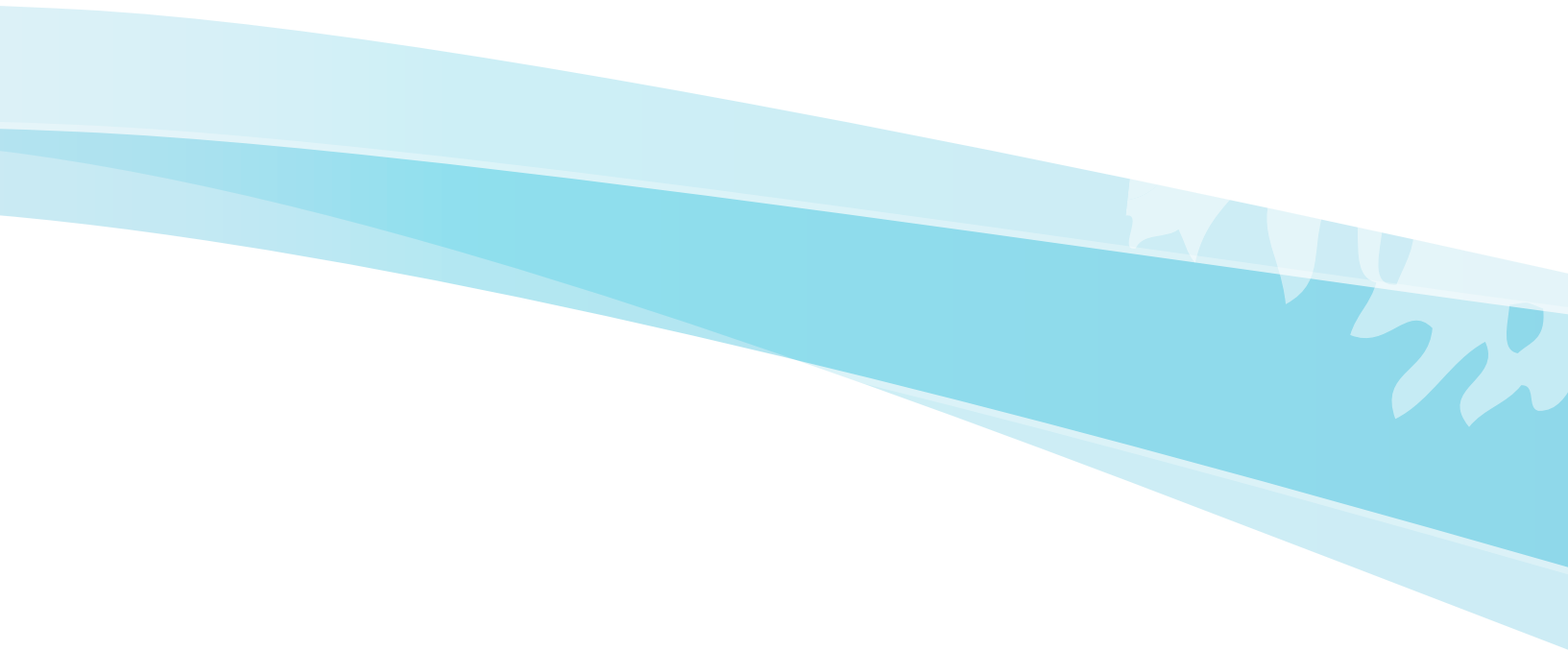
General acronym list

AGRRA:	Atlantic and Gulf Rapid Reef Assessment Program
ARC:	Australian Research Council
CARICOMP:	Caribbean Coastal Marine Productivity Program
CDHC:	Coral Disease & Health Consortium
CDWG:	CRTR Coral Disease Working Group
CRCP:	NOAA's Coral Reef Conservation Program
CRTR:	GEF's & World Bank Coral Reef Targeted Research Program
EPA:	US Environmental Protection Agency
GCDD:	Global Coral Disease Database
GEF:	Global Environment Facility
NCCOS:	National Centers for Coastal Ocean Science
NOAA:	US National Oceanic and Atmospheric Administration
NMFS:	NOAA's National Marine Fisheries Service
UNEP:	United Nations Environmental Program
USGS:	United States Geological Survey
WCMC:	World Conservation Monitoring Centre

Disease/health state acronyms

ASP:	Aspergillosis	BBD:	Black band disease
BrB:	Brown band disease	CCI:	Caribbean ciliate infection
COTS:	Crown-of-thorns starfish	DSD:	Dark spots disease
GA:	Growth anomaly	PR:	Pigmentation response
RBD:	Red band disease	SEB:	Skeletal eroding band
UWS:	Ulcerative white spots	WBD:	White band disease
WP:	White plague	WS:	White syndrome
YBD:	Yellow band disease		

appendices



Appendix 2:

Regional contact list of coral disease experts
(For a full list of experts, see www.gefcoral.org)

Caribbean

Bruckner, Andrew W. Ph.D. (CDHC)
(coral reef ecology, coral diseases, conservation)
National Marine Fisheries Service
Office of Habitat Conservation
NOAA Coral Reef Conservation Program
1315 East-West Highway
Silver Springs, Maryland 20910 USA
Andy.bruckner@noaa.gov

Gil-Agudelo, Diego L. Ph.D.
(coral microbiology)
Instituto de Investigaciones Marinas y Costeras (INVEMAR)
PO Box 1016
Cerro Punta de Betín,
Santa Marta, Magdalena, Colombia
diego.gil@invemar.org.co

Harvell, C. Drew Ph.D. (CRTR: CDWG, Chair)
(coral immunology, environmental stress, ecology)
Department of Ecology and Evolutionary Biology
Cornell University
Corson Hall
Ithaca, New York 14853, USA
cdh5@cornell.edu

Jordan-Dahlgren, Eric Ph.D. (CRTR: CDWG)
(coral reef ecology, coral disease)
Instituto de Ciencias del Mar y Limnología
Universidad Nacional Autónoma de México,
Apartado Postal 1152
77500 Cancún, Quintana Roo, México
jordan@mar.icmyl.unam.mx

Peters, Esther C. Ph.D. (CDHC)
(coral histopathology, ecotoxicology)
Tetra Tech, Inc.
10306 Eaton Place
Suite 340
Fairfax, Virginia 22030, USA
esther.peters@verizon.net

Richardson, Laurie L. Ph.D. (CDHC)
(coral microbiology)
Florida International University
Dept of Biological Sciences
11200 SW Eighth St
University Park Campus
Miami, Florida 33199, USA
richardl@FIU.edu

Ritchie, Kim B. Ph.D.
(coral microbiology)
Center for Coral Reef Research
Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, Florida 34236, USA
ritchie@mote.org

Rohwer, Forest Ph.D.
(microbiology, virology)
Department of Biology & Center for Microbial Sciences
San Diego State University
San Diego, California 92182, USA
forest@sunstroke.sdsu.edu

Santavy, Deborah Ph.D. (CDHC)
(coral microbiology, Florida Keys)
Gulf Ecology Division
One Sabine Island Drive
Gulf Breeze, Florida 32561, USA
santavy.debbie@epa.gov

Smith, Garriet W. Ph.D.
(CRTR: CDWG, Co-chair)
(microbial ecology)
University of South Carolina at Aiken
Department of Biology and Geology
471 University Parkway
Aiken, South Carolina 29801 USA
SmithRes@usca.edu

Weil, Ernesto Ph.D. (CRTR: CDWG)
(coral ecology, reproduction, disease ecology)
University of Puerto Rico
Department of Marine Science
P.O. Box 908
Lajas, Puerto Rico
eweil@caribe.net

Woodley, Cheryl M. Ph.D. (CDHC, Chair)
(microbiology, ecotoxicology)
DOC/NOAA/NOS/NCCOS
Center for Coastal Environmental
Health and Biomolecular Research
Hollings Marine Laboratory
331 Fort Johnson Rd
Charleston, South Carolina 29412, USA
cheryl.woodley@noaa.gov

Indo-Pacific

Aeby, Greta S. Ph.D. (CDHC)
(coral reef ecology, coral disease ecology)
Hawaii Institute of Marine Biology
P.O. Box 1346
Kaneohe, Hawaii 96822, USA
greta@hawaii.edu

Azam, Farooq Ph.D. (CRTR: CDWG)
(microbial ecology, microbiology)
Scripps Institution of Oceanography
University of California at San Diego
9500 Gilman Dr. La Jolla,
California 92093, USA
fazam@ucsd.edu

Jacobson, Dean M. Ph.D.
(marine ecology, coral disease, oceanography)
College of the Marshall Islands
PO Box 1258
Majuro, MH 96960, Marshall Islands
atolldino@yahoo.com

Raymundo, Laurie J. Ph.D. (CRTR: CDWG)
(reef ecology, coral disease ecology, MPAs)
University of Guam Marine Lab
UOG Station
Mangilao, Guam 96923, USA
ljraymundo@gmail.com

Rohwer, Forest Ph.D.
(microbiology, virology)
See Caribbean regional list for contact information

Vargas-Ángel, Bernardo Ph.D. (CDHC)
(histopathology, reef ecology)
Joint Institute for Marine and
Atmospheric Research
University of Hawai'i at Manoa
1000 Pope Road, Marine Science Building 312
Honolulu, HI 96822, USA
Bernardo.VargasAngel@noaa.gov

Willis, Bette L. Ph.D. (CRTR: CDWG)
(coral ecology, reproduction, disease ecology)
ARC Centre of Excellence for Coral Reef Studies
School of Marine and Tropical Biology
James Cook University
Townsville Queensland, 4811, Australia
Bette.Willis@jcu.edu.au

Work, Thierry M. D.V.M. (CDHC)
(wildlife disease, epizootiology, pathogenesis)
USGS-National Wildlife Health Center
Hawaii Field Station
PO Box 50167
Honolulu, Hawaii 96850, USA
thierry_work@usgs.gov

Red Sea/East Africa

Kushmaro, Ariel Ph.D.
(coral microbiology)
Department of Biotechnology Engineering
Ben-Gurion University of the Negev
PO Box 653
Be'er-Sheva 84105, Israel
arielkus@bgumail.bgu.ac.il

McClanahan, Timothy R. Ph.D.
(coral reef ecology, coral disease, MPAs)
Coral Reef Programs
Wildlife Conservation Society
Kibaki Flats no. 12
Bamburi, Kenyatta Beach
P.O. Box 99470
Mombasa 80107, KENYA
tmcclanahan@wcs.org

Rosenberg, Eugene Ph.D. (CRTR: CDWG)
(microbial ecology)
Department of Molecular Microbiology and Biotechnology
Tel Aviv University, Ramat Aviv 69978, Israel
eros@post.tau.ac.il

Coral Reef Targeted Research Program Centres of Excellence

Australasia Centre of Excellence
Chair: **Professor Ove Hoegh-Guldberg**, Ph.D.
Australasia Centre of Excellence
Centre for Marine Studies
Heron Island Research Station (HIRS)
The University of Queensland
Queensland 4072, Australia
Tel: +61 7 3346 9418
oveh@uq.edu.au

East Africa Centre of Excellence

Chair: **Alfonse Dubi**, Ph.D.
Institute of Marine Science
University of Dar Es Salaam
P.O. Box 668,
Zanzibar, Tanzania
Tel: +255 24 2230741/ 2232128
dubi@ims.udsm.ac.tz

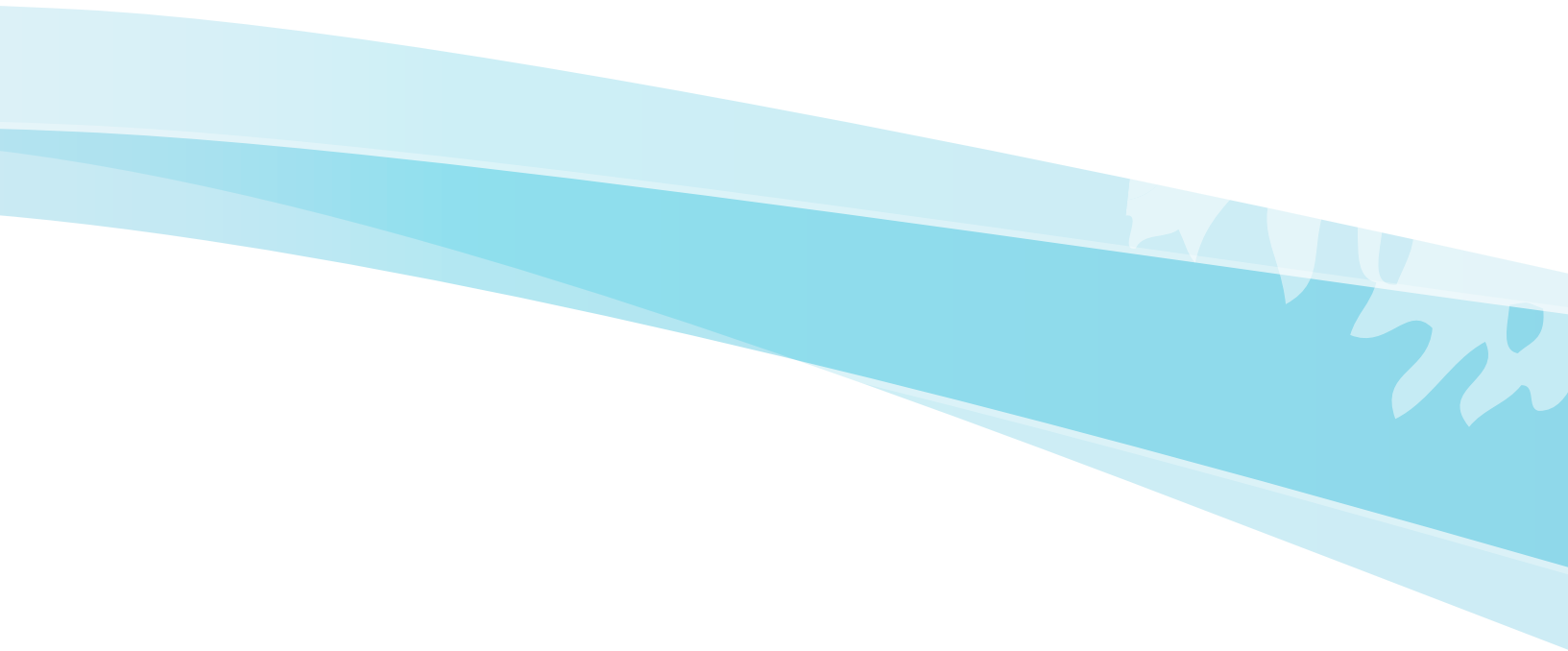
Mesoamerica Centre of Excellence

Chair: **Roberto Iglesias-Prieto**, Ph.D.
Unidad Académica Puerto Morelos (UAPM)
Instituto de Ciencias del Mar y Limnología
Universidad Nacional Autónoma de México
Apartado Postal 1152
Cancún 77500, QR
México
Tel : +52 (998) 871 02 19
iglesias@icmyl.unam.mx

South-East Asia Centre of Excellence

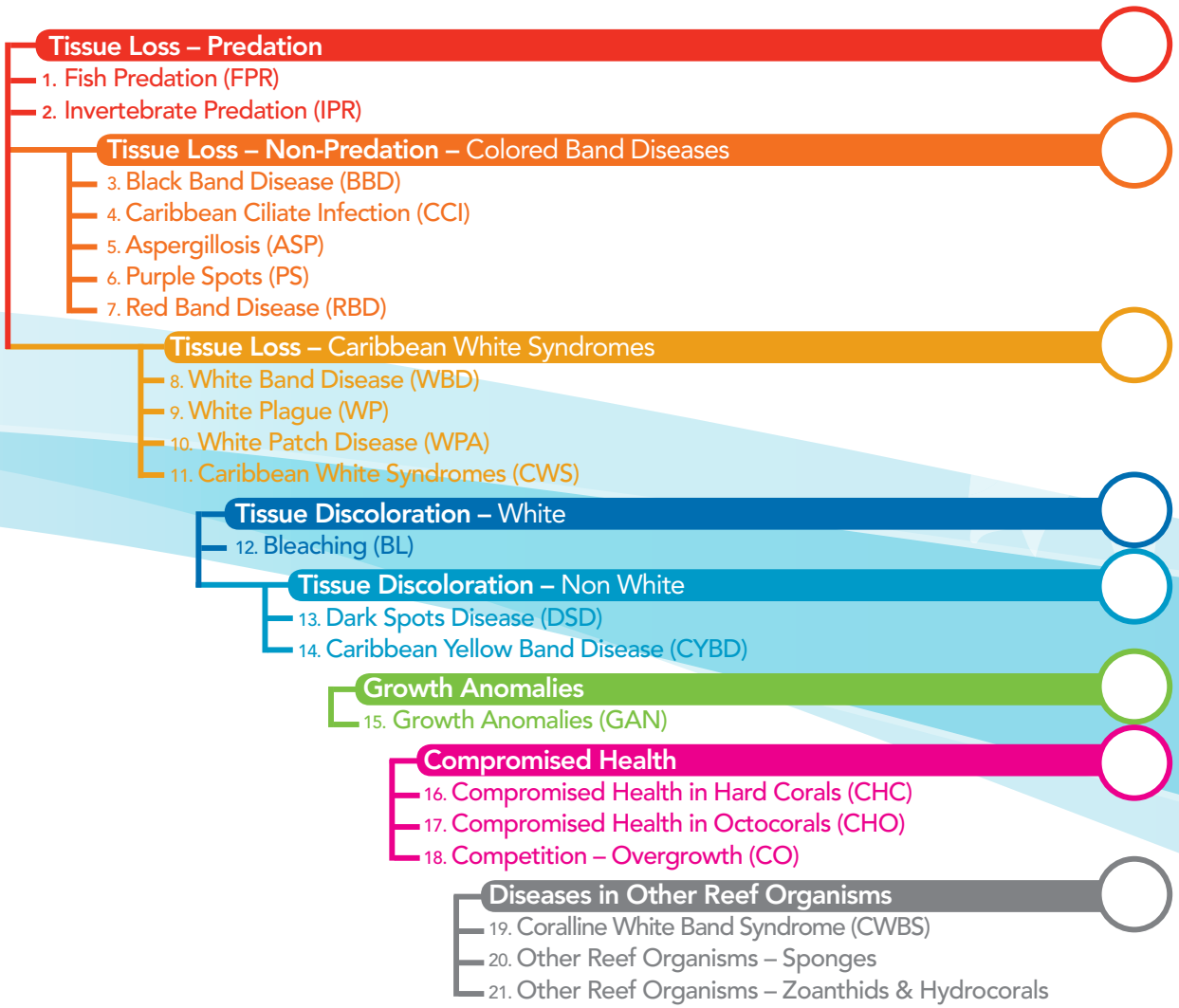
Chair: **Emeritus Professor Edgardo Gomez**, Ph.D.
The Marine Science Institute
University of the Philippines
Diliman, Quezon City
1101 Philippines
Tel: +63 2 435 7417
edgomez@upmsi.ph

appendices



Appendix 3
Indo-Pacific Coral Health – Decision Tree

Appendix 3
Caribbean Coral Health – Decision Tree

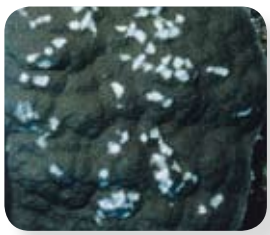


Appendix 3

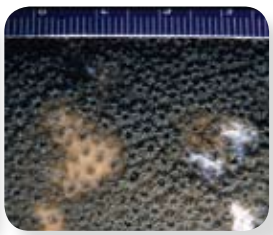
Supplementary disease and compromised health state photographs

Western Atlantic

Fish bites

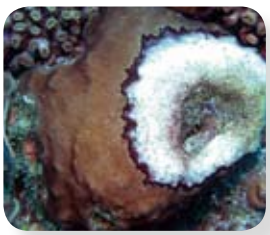


Porites astreoides spot biting



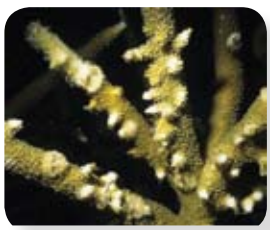
Montastraea annularis spot biting lesions lacking tissue (on right) and lesions that have begun to heal (left)

Black band disease

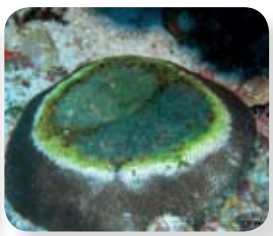


Whole colony view of *Siderastrea siderea* with black band disease

Close up of black cyanobacterial mat on *Siderastrea siderea*



Acropora cervicornis with chimney-like structures from damselfish bites



Stephanocoenia intercepta with a damselfish territory (predation), often confused with white syndrome

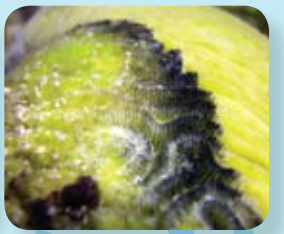
Red band disease

Meandrina meandrites with red band disease mat (arrow)

Close up of cyanobacterial mat on *Agaricia* sp.

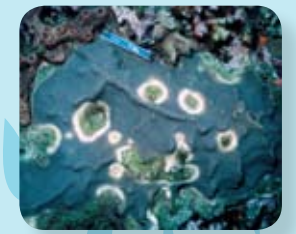
Carribean ciliate infection

Whole colony view of *Diploria labyrinthiformis* with progressing band of ciliates (arrow), dead skeleton overgrown by algae

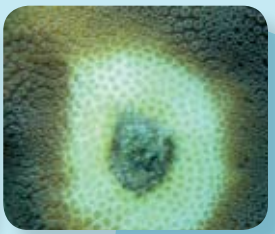


Close up of *Diploria labyrinthiformis* with band of ciliates to traveling to the right over healthy tissue

Yellow band disease



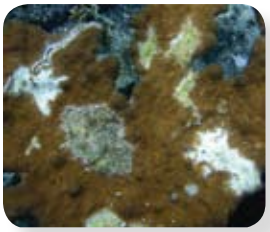
Whole colony view of *Montastraea faveolata* with multiple yellow band lesions



Close up of *Montastraea annularis* new yellow band lesion

Ciliates, *Halofolliculina* sp. from CCI mag. 50X

White patch disease



Acropora palmata with multiple white patch lesions, including acute (upper left and lower right), subacute (upper right) and an older, algal colonized lesion (middle) that has begun to heal

Close up of white patch lesion on *Acropora palmata*

White band disease

Whole colony view of *Acropora palmata* with white band disease

Close up of progressing front of white band in *Acropora palmata*

White plague

Whole colony view of *Montastraea annularis* with white plague

Close up of progressing front of white plague in *Montastraea franksi*

Dark spots disease

Siderastrea radians with dark spots disease

Close up of progression front of dark spots in *Siderastrea radians*

Carribean white syndromes

Whole colony view of *Montastraea cavernosa* with multiple white syndrome lesions

Close up *Montastraea franksi* white syndrome

Indo-Pacific/East Africa/Red Sea

Fish bites

Porites sp. with numerous parrotfish bite scars concentrated along ridges

Close up of *Porites sp.* with puffer fish bites, showing regular paired scrape marks

Gastropod predation

Acropora sp. infested with *Drupella cornus*

Close up *Acropora sp.* tissue removed by *Drupella cornus* (white region)

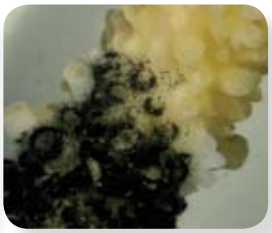
Black band disease

Whole colony view of *Coeloseris mayeri* with black band disease. The entire colony was dead one month after this photo was taken

Close up of *Echinopora lamellosa* with black band

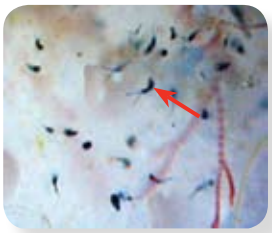
Skeletal eroding band

Acropora sp. with speckled band of ciliates. Dead skeleton colonized by algae



Close up of *Acropora intermedia* with speckled band of ciliates

Black band disease front, showing filamentous cyanobacteria adjacent to dead coral skeleton (white) on *Montipora* sp., mag. 45X



Ciliates, *Halofolliculina corallasia* (arrow) from *Acropora sp.* with skeletal eroding band, mag. 32X

Brown band disease

Acropora sp. with brown band disease (arrow)

Close up of the brown band of ciliates (arrow) on *Acropora* sp.

Acropora with brown band disease infested with ciliates

Ulcerative white spots

Whole colony view of *Porites cylindrica* with ulcerative white spots disease

Porites cylindrica showing two active lesions; the one on the left is completely devoid of tissue; the lesion on the right is bleaching, mag. 35X

White Syndrome

Pachyseris speciosa with white syndrome

Porites cylindrica with an early white syndrome lesion (arrow)

An active disease front of white syndrome spreading within *Lobophyllia hemprichii*

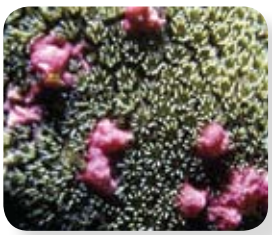
Pigmentation Response

Whole colony view of a massive *Porites* sp. pigmentation response to macroalgae abrasion

Close up of *Porites* sp. tissue swelling and pigmentation response to macroalgae abrasion

Trematodiasis

Whole colony view of *Porites compressa* with numerous trematodiasis lesions



Close up *Porites compressa* showing active pink nodules from trematodiasis

Trematode cyst (~1mm) removed from a trematodiasis lesion on *Porites compressa*, mag 40X

Growth Anomalies of a unknown cause

Massive *Porites* sp. with a growth anomaly

Massive *Porites* with a growth anomaly of a different morphology. This type appears as a white to pink plaque

Skeleton of *Porites* sp. with growth anomaly, mag. 10X

Western Atlantic data sheet template –

Colony measurements, mortality, disease prevalence and compromised health states

Recorder: **Site:** **Transect:** **Depth:** **Date:**

Species	% Live	% Mortality Old	% Mortality Recent	Size Dia	Size Height	Bleaching P/M/W	% Bleached	Disease	Predation S/W/P/D	Overgrowth S/A/T/M	Comments

Bleaching: Pale, Mottled, White. **Predation:** Snails, Fireworm, Parrotfish, Damselfish. **Overgrowth:** Sponge, Algae, Tunicate, *Millepora.*

Indo-Pacific data sheet template – Disease prevalence & compromised health states

Recorder: **Site:** **Transect:** **Depth:** **Transect Position:** **Date:**

	Total Colony Count	Growth anomaly	BBD	BrB	WS	UWS	SEB	Patchy bleach	PR
Acropora tabular									
staghorn/bushy									
bottlebrush/digitate									
corymbose									
isopora									
Anacropora/Astreopora									
Montipora									
Pocillopora									
Stylophora/Seriatopora									
Stylocoeniella/Madracis									
Porites massive									
branching									
submassive									
Goniopora/Alveopora									
Favia/Montastrea									
Favites/Echinopora									
Platygyra/Goniastrea									
Cyphastrea/Leptastrea									
Other *faviids* (genera)									
Fungids (genera)									
Galaxea/Simplastrea									
Pectinia/Oxypora									
Echinophyllia/Mycedium									
Lobophyllia/Symphyllia									
other *Mussids* (genera)									
Hydnophora									
Merulina									
Paraclav/Scapophyllia									
Pavona									
Leptoseris/Coeloseris									
Pachyseris/Gardinero									
Psammocora/Coscinarea									
Siderastrea/Pseudosider									
Euphyllia/Catal/Trachy									
Plerogyra/Physogyra									
Turbinaria/Tubastrea									
Heliopora/Tubipora									
Millepora									

Predation: **1Dr** (*Drupella*) **1Co** (*Coralliophilia*) **1CT** (COT) **1F** (Fish) *Algal overgrowth/abrasion:* **2Cy** (cyanobacteria) **2Ma** (macroalgae) **2RF** (red filamentous) *Silt smothering/abrasion:* **SI**

Indo-Pacific data sheet template – Line intercept transect data

Recorder: **Site:** **Depth:** **Date:**

	T1	T2	T3	T4	T5	T6
Acropora tabular						
corymbose						
digitate						
bottlebrush						
staghorn/bushy						
Isoporan						
Montipora						
Anacropora/Astreopora						
Pocillopora						
Stylophora/Seriatopora						
Porites massive						
Porites branching						
Porites submassive						
Goniopora/Alveopora						
Favia/Favites/Montastrea						
Platygyra/Goniastrea						
Cyphastrea/Leptastrea						
other F*aviids*						
Fungids						
Oculinids/Pectinids						
Mussids/Merulinids						
Agariicid/Siderastreids						
Dendrophyllids						
Caryophyls, Trachyphyls						
Soft corals/Gorgonians						
Heliopora/Tubipora/Millepora						
macroalgae/fleshy algae						
rock with turfing algae						
sand/silt						
recently dead standing coral						
rubble						
other (sponges, ascidians)						

Data sheet template – Lesion characterization data sheet

Recorder: **Site:** **Transect:** **Date:**

Species	Col diam (cm)	Lesion #/col	Distribution F/M/C/D/L	Location B/M/A	Lesion Diam	Lesion Color	Margin (S/I)	Severity ml/md/s/x	Timing A/S/C	Diagnosis/Comments

Distribution:

focal multifocal coalescing diffuse linear

Location:

basal medial apical

Margin:	Severity:		Timing:
Smooth	Mild	<10%	Acute (current; bare skeleton)
(S)	Moderate	10-24%	Subacute (recent; green filamentous algae)
Irregular	Severe	25-49%	Chronic
(I)	Extreme	50-100%	(epibiont community)

Table 1. Major diseases of the Western Atlantic, from published literature

Syndrome	Synonym	Host range	Presumed cause	Reference
White band disease (WBD)	White line disease, white death, white plague	*White line disease, white death, white plague*	Unkown; bacterial aggregates often present	(98)
WBD type II	WBD type II	*A. cervicornis*	*Vibrio charcharia* bacterium	(11,99,100)
White plague	Plague type II, white plague type III, plague; white band disease, white line disease	*Diploria stokesi* + 40 spp. of non-Acropora plating & massive corals	*Aurantimonas coralicida*	(31,101)
White patch disease	White pox, patchy necrosis	*A. palmata*	*Serratia marcescens*	(13,52)
Black band disease (BBD)	Black line disease	24 scleractinian corals, 1 hydrozoan, 6 gorgonians	Microbial consortium of cyanobacteria, *Beggiatoa & Desulfovibrio*	(102-104)
Yellow band disease (YBD)	Yellow blotch disease; ring bleaching, yellow pox disease; yellow band syndrome; yellow band/blotch	*Montastraea. annularis* spp. complex, other faviids ; Agaricia agaricites	Possibly *Vibrio*; may be a disease of zooxanthellae	(13,105-110)
Dark spots disease (DSD)	Dark spot disease, dark spot syndrome, Ring disease, DSD type II, Purple band syndrome, dark bands	*M. annularis M. faveolata, M. cavernosa, Siderastrea. siderea, S. intersepta, A. agaricites*	Fungal origin; associated with *Vibrio* spp.	(11,109,111,112)
Caribbean ciliate infections (CCI)	Skeletal eroding band (SEB)	>10 species including *Dichocoenia, Montastraea, Acropora*	*Halofolliculina* sp ciliate	(31,113)
Growth anomalies (GA)	Hyperplasia, Neoplasia, tumors, Gigantism, accelerated growth, chaotic polyp development, calicoblastic epithelioma	*Diploria, Colpophyllia, Porites Montastraea, Agaricia, A. palmata; Dichocoenia, Madracis*	Unknown; genetic or triggered by environmental stressors	(114,115)
Aspergillosis (ASP)	Sea fan disease	*Gorgonia* spp. & 6 other octocorals	*Aspergillus* sydowii fungus	(29,54,116,117)
Red band disease (RBD)	Red band disease type I, RBD type II	*Gorgonia, Colpophyllia, Agaricia, Mycetophyllia Stephanocoenia; Type II reported on D. stigosa, M. annularis, M. cavernosa S. radians, P. astreoides*	Microbial consortium dominated by Cyanobacteria *Schizothrix mexicana* and *S. calcicola*; second type dominated by two species of *Oscillatoria*	(104,118,119)

Table 2. Major diseases of Indo-Pacific, East African and Red Sea corals, from published literature

Syndrome	Location	Host species	Presumed cause	Reference
Black band disease (BBD)	Australia, Egypt, Fiji, India, Jordan, Papua New Guinea, Philippines, Saudi Arabia, Tonga, South Africa, CNMI, Palau	19 genera, 49 species; *Pocillopora* and *Acropora* most frequently affected	Microbial consortium dominated by cyanobacteria, sulfate-reducing bacteria and sulfide-oxidizing bacteria	(33,120-128)
White syndrome (WS)	Egypt, Australia, Solitary Islands, Philippines, Guam	Multiple spp. of *Turbinaria, Acropora, Goniastrea*,Faviidae, *Pocillopora, Porites, Pavona, Stylophora, Montipora*	Unknown	(33,34,129,130)
Ulcerative white spot disease (UWS)	Philippines, Guam	*Porites; Echinopora, Goniastrea, Heliopora, Favia, Montipora*	Possibly a *Vibrio*; normally called Porites Ulcerative White Spot Disease	(34,128,130,131)
Bacterial Bleaching	Mediterranean, Israel, Tanzania	*Oculina pategonica; Pocillopora*	*Vibrio corallyliticus, V. shiloi*	(132)
Atramentous necrosis	Great Barrier Reef, Australia	*Montipora aequituberculata*	Unknown	(133)
Growth Anomalies (GA)	CNMI, Oman , Philippines, Guam, Australia, Hawaii, Palau, Enewatak, French Polynesia, New Caledonia, Maldives, Micronesia, Marshall Islands, Japan, China	*Acropora, Pocillopora, Pavona, Fungia, Madrepora, Montipora, Platygyra, Porites, Goniastrea*	Unknown; genetic or environmental stress triggers. Also known as hyperplasia, neoplasia, tumors, calicoblastic epitheliomas	
Fungal syndrome	East African Coast	*Astreopora, Montipora, Echinopora, Acropora, Goniopora, Platygyra, massive Porites, Pocillopra, Goniastrea Hydnophora, Cyphastrea*	Unknown	(39)
Trematodiasis	Hawaii	*Porites compressa, P. lobata*	Metacercaria of a digenetic trematode (parasitic flatworm)	(138)
Skeleton eroding band (SEB)	Australia, Egypt, Jordan, PNG, Mauritius , Australia, Guam	21 genera of scleractinia; most common on *Acropora & Pocillopora*, 1 hydrozoan	*Halofolliculina corallasia*, a colonial heterotrich ciliate	(33,130,139-141)
Yellow band disease	United Arab Emirates, Arabian Gulf, Iran	4 genera, 12 species of scleractinia	Associated with a cyanobacteria	(125)
Brown band disease (BrB)	Australia, Guam	*Acropora, Pocillopora, Echinopora,* 16 spp.	*Helicostoma nonatum* ciliates	(33,130)

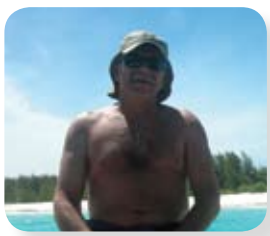
Authors

Laurie Raymundo (CDWG)
University of Guam

Courtney Couch (CDWG)
Cornell University

Andy Bruckner (CDHC)
NOAA Coral Reef Conservation Program

Drew Harvell (CDWG)
Cornell University



Ernesto Weil (CDWG)
University of Puerto Rico

Cheryl Woodley (CDHC)
NOAA Center for Coastal Environmental Health and Biomolecular Research

Thierry Work (CDHC)
USGS-National Wildlife Health Center

Eric Jordan-Dahlgren
(CDWG)
Universidad Nacional Autónoma de México

Bette Willis (CDWG)
James Cook University

Greta Aeby (CDHC)
Hawaii Institute of Marine Biology

Yui Sato
James Cook University

authors

The ***Coral Disease Handbook: Guidelines for Assessment, Monitoring and Management*** summarizes the relevant known science for managing coral disease. Produced by the Coral Reef Targeted Research and Capacity Building for Management Program and its partners, it is designed to be used in conjunction with the ***Underwater Cards for Assessing Coral Health on Indo-Pacific Reefs*** and the ***Underwater Cards for Assessing Coral Health on Caribbean Reefs***. These tools will help managers and field scientists identify and monitor infectious syndromes of coral and take the next step of implementing new management approaches.

The handbook includes three chapters on identifying infectious syndromes and their impacts in the field; a chapter on coral disease monitoring protocols; a chapter detailing methods for detecting and assessing new outbreaks of disease; and a chapter on developing new management options for coral disease. It emphasizes the synergies between infectious disease and the rapidly changing facilitators of disease outbreaks, like global warming. These factors make coral disease management a moving target requiring cooperation and knowledge exchange between microbiologists, molecular biologists, ecologists and managers.This handbook aims to integrate critical, current scientific information about coral disease to support and strengthen coral reef management.

Coral disease outbreaks have continued to increase and take out the major reef-builders in the Caribbean during the last two decades. White band hugely affected Acropora palmata in the 1970s and in the Keys we started having major impacts of black band in 1986. We are in the throes of several destructive outbreaks in the wider Caribbean now. Both in terms of coral bleaching and the residual outbreaks of coral diseases, I think the Pacific is lagging about 12 to 14 years behind the Wider Caribbean. This manual fills a critical gap in moving us to the next level in developing increasingly ambitious management approaches for coral disease.

Billy Causey, *Director*
Southeast Region, Office of National Marine Sanctuaries

Overall, it is a very impressive compilation, and is sure to be a significant contribution to progress in the field. These types of "state-of-play reviews" are invaluable, in my opinion, for capturing the state of knowledge, facilitating coordination and cooperation among researchers, and setting the agenda for future work. There should be more of them.

Paul Marshall, *Director*
Climate Change, Great Barrier Reef Marine Park Authority

The CRTR Program is a partnership between the Global Environment Facility, the World Bank, The University of Queensland (Australia), the United States National Oceanic and Atmospheric Administration (NOAA) and approximately 50 research institutes and other third-parties around the world.

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