Field Manual for Investigating Coral Disease Outbreaks

Coral Disease & Health Consortium
SOLUTIONS TODAY FOR REEFS TOMORROW

National Oceanic and Atmospheric Administration
in cooperation with Federal, State, Academic,
Non-Profit Marine Laboratories and Industry Partners

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NOAA Technical Memorandum NOS NCCOS 80

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About the CDHC

The Coral Disease and Health Consortium (CDHC) is a Working Group of the U.S. Coral Reef Task Force charged with organizing and coordinating the scientific resources of the U.S. and its territories to meet the challenge presented by globally declining coral reefs. Its mission is to preserve and protect the health of coral reef ecosystems through an understanding of the effects of natural and anthropogenic stressors on reef-building communities. The CDHC serves to unify the coral health and disease research community, identify research priorities, and encourage a new generation of coral researchers through education and outreach. The biomedical perspective and innovative technologies developed from Consortium efforts is envisioned to give scientists, resource managers, and industry new tools to identify and alleviate hidden stresses before they become environmental health crises. Currently over *125 partners,* including federal agencies, NOAA, DOI, EPA, along with academia, non-profit and industry, contribute their time and expertise to the CDHC, while organizational infrastructure is supported by the congressionally funded NOAA Coral Reef Conservation Program.

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PREFACE

Coral reefs throughout their circumtropical range are declining at an accelerating rate. Recent predictions indicate that 20% of the world's reefs have been degraded, another 24% are under imminent risk of collapse, and if current estimates hold, by 2030, 26% of the world's reefs will be lost (Wilkinson 2004). Recent changes to these ecosystems have included losses of apex predators, reductions of important herbivorous fishes and invertebrates, and precipitous declines in living coral cover, with many reefs now dominated by macroalgae. Causes have been described in broad sweeping terms: global climate change, over-fishing and destructive fishing, land-based sources of pollution, sedimentation, hurricanes, mass bleaching events and disease. Recognition that corals can succumb to disease was first reported in the early 1970's. Then it was a unique observation, with relatively few isolated reports until the mid 1990's. Today disease has spread to over 150 species of coral, reported from 65 countries throughout all of the world's tropical oceans (WCMC Global Coral Disease Database). While disease continues to increase in frequency and distribution throughout the world, definitive causes of coral diseases have remained elusive for the most part, with reef managers not sufficiently armed to combat it.

Wobeser (1994) writes, *"Disease management is a tactical battle in which one uses intelligence gathered about the disease to identify the most vulnerable point at which to attack"*. Understanding the disease process and how it relates to ecology are the necessary steps when attempting to determine causation. The rationale, however, for studying coral disease is often challenged as an esoteric pursuit that *'you can't do anything about anyway'*. This myopic point of view undermines the 'intelligence gathering' efforts. But it is this 'intelligence' that makes it possible to assess the nature and significance of the disease, and in turn identify management strategies (*'points at which to attack'*) to restrict or curb the occurrence or effects of the disease.

There is a recognized need, first put forth in the CDHC National Research Plan (Woodley et al. 2003), articulated by resource managers, and highlighted in several Local Action Strategy plans, to establish local response capabilities to investigate coral disease outbreaks. Establishing regional Outbreak Investigation Response Teams addresses this need by providing a network of well trained responders that can be mobilized on short notice to carry out formal investigations into unusual occurrences of coral disease or mortality.

This document was created through the collaborative effort of members of the CDHC to provide standardized protocols and procedures for field investigations of coral disease outbreaks. The authors also gained insight and inspiration from other field manuals published for wildlife (Friend 2006; Friend and Franson 1999) and marine mammals (Geraci and Lounsbury 1993). The framework for responses is set up such that it is consistent with practices of veterinary and wildlife disease experts to enable communication with and gain support from these disciplines. Field operations provide a

critical link in disease diagnostics. Consistent and efficient sampling and data collection are crucial to effective laboratory analyses and ultimately to the diagnosis of a disease. This manual is intended to serve as an operational guide to coordinate effective, informative responses by outbreak response teams to unusual incidents of coral disease or mortalities. As such, there are chapters intended to assist in gathering quality information and maintaining specimen integrity, both of which are needed to develop a reliable diagnosis. We have provided a summary of ecological and environmental information that should be collected in the field during an outbreak response to assist in developing a diagnosis. Outlined, in general and in specifics, are collection techniques, preservation methods for different analyses, and shipping procedures. Universal precaution measures when dealing with diseases (i.e., work from clean to dirty areas), biological containment and disinfection regimes have also been highlighted. We have attempted to point out critical control points in each of the procedures or methodologies that must be adhered to minimize the risk of compromising the samples or biasing further analyses. Each investigation will, of course, have its own unique features, requiring that some flexibility be incorporated into the field operation. The initial steps in a Coral Disease Outbreak Investigation invariably occur in the field and thus a cohesive management scheme, including operational pre-planning, is critical to achieve success. To provide continuity, structure and consistency, the Incident Command Structure (ICS) was adopted as the standardized emergency management strategy for Coral Disease Outbreak Investigations, and adapted from that used by all other U.S. agencies operating under the National Interagency Management System (NIMS).

This manual was developed as an aid to provide context for outbreak investigations and to help train coral disease outbreak response teams so that coordinated response operations can be executed. Chapter 1 provides a rationale for the need to study coral disease and respond to disease outbreaks. Chapter 2 identifies elements critical in the advanced planning process and includes issues such as regulatory and permitting authorities, criteria for mounting a response, and logistical considerations. Chapter 3 is dedicated to describing ICS structure, the functional roles and responsibilities of response team members, and its operation as it has been adapted to Coral Disease Outbreak Investigations. Chapter 4 focuses on the methodologies for collecting field data, samples and preservation techniques to preserve sample integrity suitable for laboratory analyses. Since coral disease field investigations by their very nature requires underwater operations. Chapter 5 addresses safety precautions on the boat, by response divers and during field laboratory operations.

Cheryl M. Woodley

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Chapter 1

Perspectives on Coral Disease and Outbreak Investigations

1.1 The Issue

Found in seas of over 100 countries, coral reefs cover an estimated 284,300 km^2 (ICRIN 2000b). Per unit area, they are one of the World's most valuable ecosystems in terms of ecological, economic and cultural resources, yet coral reefs are among the world's failing ecosystems. We are losing them at an accelerating rate (Wilkinson 2002). Recent predictions indicate that 58–70% of coral reefs globally are directly threatened by human-associated activities (Bryant et al. 1998; Goreau et al. 2000; Hoegh-Guldberg 1999; Wilkinson 1999), while over 80% of the Caribbean coral-reef cover has disappeared in the last 30 years (Gardner et al. 2003). According to estimates in 2004, 20% of the world's coral reefs have already been degraded beyond the potential for recovery, 24% are under imminent risk of collapse, and another 26% are under a longer term threat of collapse (Wilkinson 2004).

In both terrestrial and marine systems, wildlife disease outbreaks and mass mortality are recognized as important indicators of ecological disturbances. The role of diseases in regulating a species' survival has escalated due to environmental changes such as 1) alterations in habitat (e.g., fragmentation or loss, pollution, climate change); 2) shifts in populations (e.g., introduction of new species; change in predator/prey relations); and 3) changes in disease ecology (e.g., loss of endemic stability; virulence and pathogenicity of agents; density of susceptible hosts) (Deem et al. 2001; Morner et al. 2002).

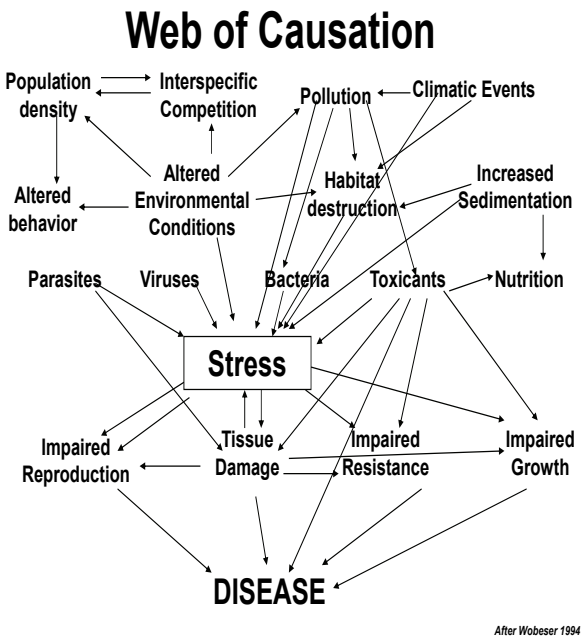
Similarly, coral reef declines are viewed as sentinels for a degraded ocean condition. The causes have only been described in broad sweeping terms implicating coastal urban and industrial development, agricultural runoff, sedimentation, overfishing, marine pollution, climate change and disease (Bellwood et al. 2004; Bryant et al. 1998; Risk 1999; Turgeon et al. 2002; Walker and Ormond 1982). These threats have contributed to losses of apex predators, removal of key herbivorous fishes and invertebrates, precipitous declines in coral cover as corals become stressed and die, and ecosystem shifts to dominance by macroalgae on reefs and similar disruptions in other ocean systems.

Coral disease is manifested in a number of different forms from acute mortality leading to a rapid loss of diversity and abundance, to chronic partial mortality resulting in progressive tissue loss, with non-acute, sub-lethal effects. The outcome of various disease states may result in reduced growth, reduced reproductive effort and recruitment, increased incidence of various coral disease conditions and mortality, ultimately cascading into ecosystem deterioration (CRMP 2001; Hoegh-Guldberg 1999; Knowlton 2001; Nystrom et al. 2000; Patterson et al. 2002; Porter and Tougas 2001; Richmond 1993). Coral disease whether infectious or noninfectious in nature is a significant challenge to conserving and protecting coral reefs. Definitive root causes to these

diseases, however, remain elusive and the increasing frequency and distribution throughout the world pose major threats to reefs and challenges to reef managers to combat these threats.

> Health is relative occurring along "…. a continuum between two endpoints: absolute health (a state in which all functions are optimal) and death, which occurs when functions are so severely compromised that life is impossible. Between the two points there is a region of relative health that blends imperceptibly into a region that we can define as disease." (Wobeser 2006)

1.2 Defining Coral Disease



It is tempting to assume that coral "disease" refers only to clearly visible signs of infection by a pathogen; however, disease can be caused by abiotic factors as well as a response to biotic stressors. We have adopted the definition of Wobeser (1981) that states disease is *'any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects, or combinations of these factors'* to characterize disease in its full meaning. For diseases of coral, we know that in addition to biological agents, many other risk factors exist, such as climate change, environment degradation, toxicants and physical damage. Therefore, it is more appropriate to consider coral diseases to result from a *'web of causation'* with many factors and co-factors ultimately contributing to disease (Wobeser 1994), rather than one distinct agent. To determine causation a more holistic perspective must be adopted that includes the host, the agent, and the environment. In the case of coral, this includes the animal, plant symbiont and microbial flora, collectively referred to as the holobiont (Wegley et al. 2004).

Diseases of stony corals and gorgonians can fall into several categories:

- **Bleaching -** loss or degradation of zooxanthellae due to biotic (bacteria) or abiotic (e.g., temperature, UV radiation, salinity, toxicants) causes

- **Non-infectious diseases –** physiological and morphological (e.g., tissue loss or discoloration) changes due to agents such as toxins or toxicants, sedimentation, pollution, and other environmental stressors

- **Trauma –** physical damage (e.g., groundings, fish bites, snail predation)

- **Parasitic infections** – infestation by protozoans (e.g., ciliates, amoeba), metazoans (e.g., trematodes, flatworms, flukes) or parazoans (e.g., sponges)

- **Growth Anomalies** - abnormal growth and development, including hypertrophy, hyperplasia, neoplasia, tumors

- **Infectious diseases -** partial and whole colony mortality caused by bacteria, fungi, viruses and other microorganisms

1.3 Why Study Coral Disease?

The short answer is to determine the cause(s) in order to identify management options that can help control disease or mitigate its impacts to protect corals more effectively.

Disease can cause significant effects on the ecology, structure and function of coral reef ecosystems. In the Caribbean, coral disease has caused extensive losses of living coral cover, shifts in coral community structure, and extirpations of certain key reef-building species. For conservation to be effective, it is imperative not only to understand biogeographical patterns, community structure, population dynamics and individual behavior (i.e., biology) of the host species (Deem et al. 2001), but also the threats affecting these species and the health effects (i.e., consequences of the threat). Only when health parameters are incorporated into conservation models and synthesized into knowledge, will managers have more robust management options. For stony corals, basic information on biogeographic distribution and community structure is known, baseline assessments of biodiversity, population dynamics, and cover have been completed in representative locations, and the broad threats impacting reef communities have been identified. However, specific data on the prevalence and impacts of diseases and relationships between coral mortality and other stressors are available for relatively few locations. A handful of the better known diseases have been only partially characterized, and effective management responses are currently unknown. Too often the study of coral disease is viewed more as an academic exercise rather than an avenue to developing successful conservation measures. In fact, local management agencies often view diseases and bleaching as a global problem that is "*just a normal part of life that we can't do anything about anyway"*. As we look at the scope of disease effects----"*any*

impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxins, and climate; infectious agents; inherent or congenital defects, or combinations of these factors" (Wobeser 1981) it is the organism's failure to resolve these 'impairments' (or injury), at the cellular physiological or tissue level that results in the dysfunction we recognize as disease. These dysfunctions then are driving the decline of corals (and other wildlife) by affecting individual responses, population dynamics, community structures and biogeographical patterns (Deem et al. 2001). In other words, the long-term existence of corals and their ability to fulfill their ecological roles are being compromised by disease. It is critical that health and disease are no longer allowed to be a limiting factor in coral conservation efforts. Therefore, every effort should be made to understand the factors controlling coral reef health conditions and the impact of diseases on coral reef system dynamics, and incorporate this information into the construction of conservation programs.

The marked increase in disease incidence, the severity of the impacts being observed, and 'signatures' of recently emerged coral disease were not documented before the 1990's. Together, these changes mark an alarming threat to coral biodiversity, ecosystem stability and sustainability of reefs for the future (Daszak et al. 2001). To understand the causes of disease and their significance, and to identify control and management measures will require a broad integration of relevant disciplines that include health specialties (i.e., veterinary and medical sciences, pathology, medical microbiology, toxicology, epidemiology) together with ocean sciences (i.e., wildlife and marine ecology, marine biology, oceanography), basic sciences (i.e., biochemistry, cell physiology, microbiology, toxicology) and social and economic sciences involving those who help interface with the public and politicians (i.e., resource managers, sociologists, economists).

The gaps in our understanding of many factors affecting coral health are vast. Most coral diseases have no known etiology nor have the diseases been rigorously classified. There is little in the way of diagnostics or field tests for disease surveillance. Investigations of coral diseases often occur without guidance from veterinary scientists, cell physiologists, toxicologists or epidemiologists. A valuable shift in how coral disease is studied would be the application of the integrated principles of epidemiology and risk analysis to coral health assessments. An epidemiologist is usually not trained in one specific discipline, but is *"a master of 'lateral thinking', trying to see connections between what are probably isolated observations of completely different natural phenomena"* (Halpin 1975 as cited by; Wobeser 1994). Epidemiology is a powerful tool that can identify predictors (risk factors) for changes in coral health and ecosystem condition, quantify the strength of those associations, and focus diagnostic efforts toward identifying etiology. Since most disease in coral is likely to be multi-factorial, identification of risk factors and use of ecological risk assessment methodologies can direct and prioritize management strategies toward risk reduction without requiring knowledge of specific etiologies. While risk assessment is a process that assigns probabilities to adverse effects of human activities or natural damaging events, it does not address health assessment which is concerned with determining the occurrence and causes of impairments of nonhuman populations and communities, a field known as ecological epidemiology (Suter 2006). Thus, integrating ecological epidemiology (biological assessment and causal analyses) with risk

assessment (risk models that link alternative decisions to future conditions) provides a systematic means to improve an understanding of the causal chain of events, identifying factors on a quantitative basis for informed management decisions (Suter 2006) and a logical, systematic approach to understand the complexities of disease. Investigations require astute observations and critical thinking drawing on many disciplines and types of information to develop quantitative comparisons among groups and various factors. The synthesis of this information can then be used to solve one of three basic problems: causation, significance or control (Wobeser 1994).

Our goal in studying coral disease is ***conservation*** of our world's reefs, but to do this we must be able to:

- ***Describe*** new diseases
- Determine ***causation***
- ***Identify the source*** of current outbreak
- ***Determine risk factors*** and ***conduct risk analysis*** for informed decision making
- Implement disease-specific ***surveillance*** measures
- ***Evaluate existing prevention/control*** measures
- ***Reduce risk*** of future outbreaks

Effective management of coral health will require being equipped to recognize new and reemerging infections, non-infectious disease conditions, and understand the factors involved in disease emergence, prevention, and elimination. This requires adopting a methodology appropriate for assimilating and synthesizing numerous and diverse data, such as those developed in the fields of risk analysis and ecological epidemiology. The broad areas that have been shown to influence emergence of disease include: 1) microbial adaptation and change, 2) human demographics and the consequences of that behavior, 3) technology and industry, 4) economic development and land-use practices, 5) international travel and commerce, and 6) the breakdown of health measures. In summary, we first need to study this major problem in a detailed, standardized manner and share the findings with the research and resource management communities.

1.4 Why Establish a Response System for Coral Disease Outbreaks?

"Stopping investigation in the here and now, will leave you vulnerable to why and how!"
(AAZV 2008)

An outbreak is commonly defined as an unexpected increase in disease or mortality in a time or place where it does not normally occur or at a frequency greater than previously observed. For coral, an outbreak may also be defined as disease occurring in a particular

species of interest or manifesting signs not previously described. Outbreaks are usually transitory and short-lived and should be treated with a matter of urgency to collect as much information as possible while it is available. In contrast, insidious, chronic diseases can have equally devastating effects on populations and communities, yet their covert nature make them hard to detect and difficult to garner support for an investigation. Nevertheless it is critically important to investigate. Developing a Response System to investigate coral disease outbreaks provides the opportunity to methodically collect a range of data to assist in determining its significance, epizootiology and causal linkages, to test the adequacy of wildlife disease protocols to diagnose the principal cause, and to evaluate the findings, develop prediction models and present options for future research and mitigation to resource managers in a timely manner.

Outbreak investigations are designed to determine the extent and impact of the event, causative agent(s) and its reservoir or source, and transmission routes. They can also be used to identify knowledge gaps, help formulate hypotheses for further study and focus research goals, and help identify control or management strategies. Outbreak investigations are most important when almost nothing is known about the disease(s) (as is the case for most coral diseases) or when a new disease is discovered. An organized, systematic approach helps create both clinical and diagnostic case definitions, identify risk factors, and formulate hypotheses to target control and management strategies. When the cause of an outbreak has been clinically determined by identification of a known pathogen (e.g., white plague II), but the source (reservoir) or route of transmission remains unknown, there often remains much investigative work to be done (as was the case with *Vibrio shiloii,* the causative agent of one type of bacterial bleaching). Investigations can then focus on filling the knowledge gaps in the ecology of the disease to better guide future control and management efforts. Therefore a Response System, using a standardized approach helps answer the 'big picture' questions:

- **What is it? (well recognized or emerging disease?)**
- **What species are being affected in the area?**
- **What species are NOT being affected in the area?**
- **Where did it come from? (reservoir)**
- **How is it spreading? (transmission)**
- **How common is it? (prevalence)**
- **What impact is it having on affected species and populations? (effects)**
- **How can you control it? (stop spread)**
- **What risk factors (i.e., biological, chemical or physical) are co-occurring with the disease? (prediction)**
- **What about future management?**

1.5 Anatomy of an Outbreak Investigation

(from Pavlin 2003; Reingold 1998; Wobeser 1994)

There are 10 basic steps that are common to most disease outbreak investigations:

- **Establish the existence of an outbreak and develop case definitions. [Epidemiological Investigation]**

This is the same as starting any case description with the signalment (i.e., description of distinguishing features, signs, and information related to the organism being examined) and history of the animals and/or group of animals. This should include information such as their environment, proximities to potential contaminant sources or recent activities or weather or climatic events that may contribute to the outbreak. The case definition is a standard set of criteria applied to arrive at an initial, preliminary diagnosis and determine whether the reported information meets the criteria for an outbreak.

- **Establish endemic level (background rate) of disease**

Determining if the disease occurrence or prevalence is higher than background levels, spatially (i.e., for that particular location, reef or habitat) and temporally (i.e., for the specific time period when the presumed outbreak is reported) is a key to defining 'unusual'. This can only be accomplished through disease surveillance (e.g., monitoring of seasonal trends in abundance). This type of information is rarely available for coral disease.

- **Characterize the outbreak in terms of who, what, when and where.**

The objective is to identify common factors that are associated with the disease that don't occur when and where the disease is absent. Thus it is important to standardize collections in terms of data and types of samples because developing causal links may take weeks to years.

- **Examine the descriptive epidemiological (or epizootiological) features of the case.**

Descriptive epidemiology involves determining the number of cases and mapping them to determine the distribution of cases in space and time and contrasting this with past events, including cross species comparisons with life stages and associated environmental factors. This information can provide valuable leads as to the source or nature of the agent or routes of exposure.

- **Generate tentative hypotheses.**

A tentative hypothesis is developed to explain the most likely cause(s), source and risk of spread of the cases.

- **Test hypotheses**

Once the hypothesis is generated it can be evaluated against the known facts about the potential agent(s) (i.e., analytical epidemiology). The goal is to assess the relationship between a given exposure and the observed disease, determine if it is

statistically significant and biologically meaningful. Several iterations of hypothesis development may be required.

- **Collect and test environmental and biological samples [Environmental Investigation]**

The findings of the 'epidemiological investigation' should guide the collection and testing of specimens and samples. The basis for these analytical tests is to compare affected and non-affected populations. These new data are added to previous data and information to accept or reject the prevailing hypothesis and develop a new one if rejected. This is essentially the differential diagnostic process.

- **Confirm or verify the diagnosis and the cases are 'real'.**

This is not necessarily immediate. Review clinical and laboratory findings for consistency and confirm or reject suspected diagnosis based on these analyses.

- **Prepare a written report**

It is important to summarize the investigation in a report that includes the reason for the investigation; general characteristics of the investigation, the clinical descriptions, results, conclusions on the nature of the disease, source of outbreak and method of transmission, and any possible recommendations for control or management.

- **Implement control/management strategies**

Disease management strategies for coral reefs must recognize the three basic determinants of disease: the host, the agent and the environment. The key to effecting disease outcomes is through successful manipulation of disease determinants and management or mitigation of related human impacts.

Chapter 2

Getting Organized – Advance Planning

2.1 Coral Disease Investigative Response System: Goals & Objectives

The goal of a Response System is to facilitate the investigation of coral disease outbreaks that are unusual in nature by providing a framework of operation that promotes a logical, systematic collection of information and samples, sufficient to allow the formulation of a hypothesis to explain why the outbreak occurred (Wobeser 1994).

The objectives of an Outbreak Investigation System are to:

- Provide **field personnel trained** in investigative techniques to ensure proficiency in survey, collection and processing techniques
- Encourage **Responder awareness** of the need to exercise *Critical Observing* (i.e., objective rather than subjective, using validated classification schemes, being aware of problems associated with over-interpretation of vague signs and of bias from prior information) and *Critical Thinking* (ability and willingness to seek both contradictory as well as confirmatory information when collecting evidence and make objective judgments based on well documented information) throughout the investigation.
- Adapt **Incident Command System** components to provide an incident management and procedural framework for conducting a coral disease outbreak investigation
- Execute an organized, systematic approach to collect **relevant epidemiological and environmental data, and samples** for developing clinical and diagnostic case definitions
- Formulate **hypotheses** as to the cause of the outbreak
- Create a **database** of disease information for retrospective and prospective investigations
- Identify **knowledge gaps**
- Formulate **hypotheses for further studies** and focus research goals.
- Provide information to help identify **disease management and control strategies**.

2.2 Regulatory Authority

The Response System and all of its members, including the investigative response team must function within the legal structure of the jurisdiction overseeing the area affected by the outbreak. This may be federal, state, regional and/or local authorities, and in some cases (e.g., when working with ESA listed corals) NOAA/NMFS and U. S. Fish and Wildlife Service, for example, collection of coral in all U.S. jurisdictions requires permitting by the local governing agency(s). Permits may also be necessary for surveys, tagging and other activities associated with the response. It is important to identify local authorities, such as Sanctuary and Park managers and establish partnerships to involve them early in the response process. The assistance of these individuals often is critical in expediting permitting and providing logistical support for their specific areas.

2.3 Organization of Response System

Every response, no matter the size, needs an organized protocol of response. The CDHC Response System is a tiered decision process coordinated through a National Center with input from an Expert Working Group and in collaboration with the Regional Coordinator. The initial phase of a response begins with an observation by a diver of a situation considered unusual and involves diseased coral which is reported to the CDHC. This is followed by notification though the Response network to the area's Regional Coordinator (Chapter 3 and Level I Report; Appendix II). A series of events are then initiated that includes: 1) evaluation of the report by experts; 2) identification of possible responses (Chapter 3 and Level II or Level III; Appendix III & IV); 3) recommendation of possible steps to reduce spread (i.e., quarantine); 4) further assessment of the situation to identify conditions that may aggravate or mitigate the event; and if warranted; 5) mobilization of a Response Team to document and characterize the incident, and collect and stabilize samples for further analyses. These steps may be moved through more quickly, if for example, an experienced research diver familiar with disease is the original observer (e.g., a Level I report could directly trigger a Level III response).

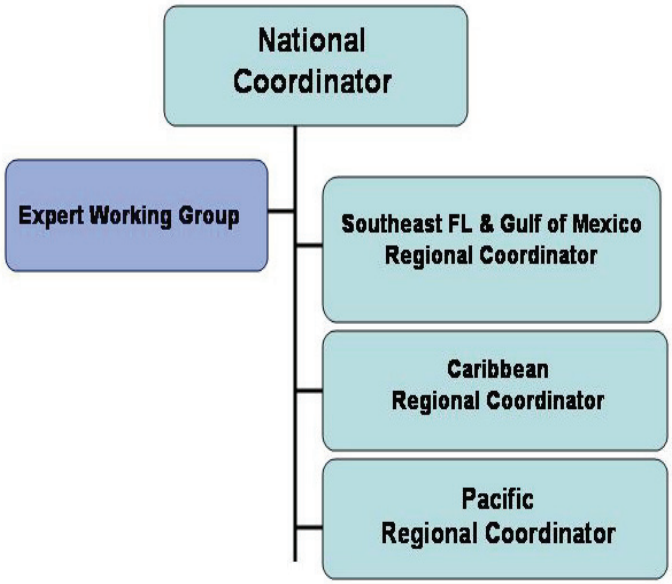


Figure 2.3 Organizational Chart for U.S. Response System.

The implementation of the Response System is through Regional Response Coordinators and a network of responders. Minimally, a lead Regional Coordinator is appointed for each region of the U.S. (Fig. 2.3), with additional Coordinators as appropriate in specific

jurisdictions. Each Response Team includes an Incident Commander (who may also be the Regional Coordinator), a Survey Team, Collection Team and Support Team. Ideally, various members of the Response Team are trained in multiple tasks and can participate in all activities as necessary.

The **essential members** of a response network include:

- An Incident Commander responsible for coordination of all field activities
- An Outbreak Investigation Response Team with a complement of scientists responsible for the collection of field data and samples, and on-boat processing of samples
- Access to veterinary consultations and scientists able to conduct specialized sample collection if necessary
- Local ecological and logistical experts (may be part of the Response Team) able to assist with coordination and knowledgeable about relevant related environmental and ecological parameters
- Public relations specialist
- A set of labs with a compliment of specialty diagnostic or analytical procedures for sample processing and analysis

A specific outbreak investigation will be led by an Incident Commander (IC) under an Incident Command Structured response. The IC will make immediate recommendations to the coral disease outbreak network on how to proceed with response activities.

2.3.1 National Coordinating Center

The primary responsibility of the National Coordinating Center (NCC) is to provide a centralized location for receiving and verifying coral disease outbreak reports and to coordinate the appropriate response. The NCC should also:

- Provide training to response team members and volunteers
- Provide supplies and equipment for surveys and response kits
- Serve as Liaison Office to notify and work with other federal and local authorities.
- Maintain current files on the capabilities of each region i.e., response team members, logistical support, emergency care facilities, and laboratory diagnostic capabilities.
- Maintain communication among all investigative team members
- Track samples sent to authorized laboratories or individuals for analyses
- Gather and archive data

- Review and assist in development of reports and identification of recommendations
- Report findings to appropriate government agencies

2.3.2 National Coordinator

The National Coordinator serves as a central contact for all Regional Coordinators and collates all reports of verified Level I observations and Level II investigations submitted by Regional Coordinators. The National Coordinator will convene the Expert Working Group to make decisions in cases that suggest the need for a Level III Response. The National Coordinator also works directly with the Regional Coordinator to ensure reports are produced in a timely manner and provides analysis and recommendations to the managers. (See sections 2.4 and 3.6 for description of response decision process)

2.3.3 Expert Working Group

The Expert Working Group includes individuals with knowledge and experience in coral diseases and pathology to provide guidance for (i) developing and implementing the contingency plan to assist in responding to unusual coral disease outbreaks; (ii) assists in determining whether an unusual coral disease outbreak is occurring and the need for a Level II or Level III response; and (iii) assists the National Coordinator in determining, after an unusual coral disease investigation response has begun, when response actions with respect to that incident should be terminated.

2.3.4 Regional Coordinators

The regions have been nominally designated based on the location of U.S. coral reefs, history of outbreaks or high prevalence of new or emerging disease and logistical considerations. Initially, there will be one Regional Coordinator designated for Florida and the Gulf of Mexico, the U.S. Atlantic (Puerto Rico and USVI), Hawaii, and the U.S. Pacific (American Samoa, Guam and CNMI). Regions will be added (e.g., Freely Associated States) and subdivided into jurisdictions as responders are available and as needed.

The Regional Coordinator is responsible for the overall communication and logistics for a given Outbreak involving an Incident Response. This includes such activities as:

- Incident Coordination
- Logistics
- Safety and permitting issues
- Determining amount, type and quality of data appropriate to collect

- Initial threat assessment
- How to implement control and prevention procedures
- Transmitting data to a centralized data analysis facility
- Communication with scientists, managers and the public.
- Preparing summary reports

Regional Coordinators should take steps well in advance of an Incident to identify and train Response Team members, compile lists of support services and contacts as well as maintenance and replenishing sampling kits. A list of suggested equipment is provided (Appendix VI), recognizing equipment needs will vary based on location, species affected, and availability.

The Regional Coordinator (RC) is notified when an Incident Report is received (either through the National Office or directly). The RC conducts interviews with those submitting the report to complete Level I information needs (Level I Report form, Appendix II) and contacts the National Coordinator (NC) to report findings and recommendations on whether the case is resolved or if a Level II response (Appendix III) is necessary. After review of the Level I report, if a Level II response is activated, the RC organizes and conducts the Level II response in collaboration with the designated Incident Commander, and reports out on findings to the NC. The RC also participates in the Working Group Consultation to determine the need for a Level III response (Appendix IV).

Once a Level III response is activated, Regional Coordinators, in collaboration with the Incident Commander and Response Team, are responsible for: 1) conducting a preliminary assessment of the outbreak event (including sample collections, if warranted) and notifying management agencies and other appropriate stakeholders of the status; 2) evaluating the seriousness of the outbreak and classifying the threat (i.e., What impacts to the reef ecosystem will result from the outbreak on a local, regional, or national scale?); 3) assessing the feasibility of containing the disease and reducing any contributing anthropogenic stresses (i.e. chemical and thermal inputs); 4) providing recommendations to decision-makers regarding potential response; and 5) providing guidance for efficient control methods.

2.3.5 Media and Public Affairs Official

Unusual outbreaks of disease among coral reefs can become a hot news topic particularly if diving is restricted by quarantining an affected reef. Nearly everyone is influenced by the media; therefore it is critical that the information given by the Response Network is accurate and consistent among responders, and does not extrapolate beyond the facts. Each Region and responding Agency should have a protocol for interacting with the media. It is important to become familiar with the local protocols and contacts. It is recommended that each Regional Coordinator identify a Public Affairs Official who is

familiar with such protocols, and who will be responsible for reporting the progress of the investigation to local authorities and managers, as well as interacting with the press to make public notification as needed.

2.4 The Decision Process

The response process for a coral disease outbreak investigation is essentially a triage that is initiated by a report from the field (i.e., public, research or recreational diver) to a Response Network contact. Public notices providing contact procedures and reporting forms should be widely distributed to commercial dive shops, local management agencies and marine patrols. Consultations with area experts verify the report and determine the sufficiency of information for decision-making. If the report is valid and information is insufficient, then additional data is obtained to determine the existence of an outbreak. If an outbreak is confirmed then a full response conducted under ICS guidelines is initiated and the Response Team is deployed to the field.

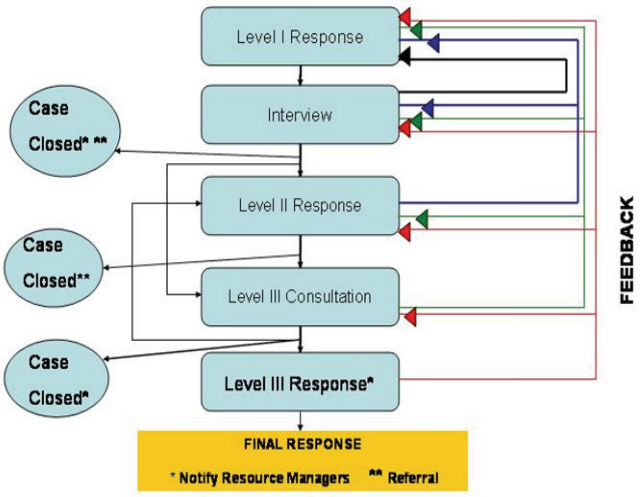


Figure 2.4 Response process, decision points and information flow.

2.5 The Decision Criteria

Until information can be assimilated to accurately diagnose disease and sufficient surveillance has been conducted to determine prevalence and incidence rates it will be difficult to discriminate between enzootic and epizootic diseases. Therefore initially, several reports identifying an event, photographic documentation, and preliminary survey data (Levels I and II) may be needed to determine whether a Level III response is appropriate. The guidelines for making such decisions include:

- Does the incident represent an unexpected increase in disease or mortality in a time or place where it does not normally occur or at a level that cannot be explained? Or in a species in which it has never been reported?
- Is the frequency of occurrence or extent of mortality greater than previously observed?
- Is the disease affecting a particular species of interest?
- Have the manifesting disease signs been previously described?

- Can the cause of the event be readily determined (e.g., major hurricane, oil spill)?

2.6 The Response Team

The **essential elements** of a response include an incident commander and an outbreak investigation response team with a complement of scientists capable of collecting, processing and analyzing samples and data. The composition of the response team will depend on the extent and location of any given outbreak. Different strategies will be required in any given situation and it is vital to distinguish between critical elements in the procedures and where flexibilities are acceptable. Common to all responses is the need to:

- Respond rapidly
- Communicate with local authorities (most often resource management offices) before, during and after a Response
- Evaluate the situation
- Implement the Incident Action Plan
- Ensure safety for the team
- Transport samples and data to designated labs and National Coordinating Center in a timely manner
- Provide relevant information to Public Affairs Officials
- Maintain Communications with the operations center and National Coordinating Center

2.6.1 Responsibilities

The core team requires a wide range of expertise. Foremost is the need for an individual that can organize others, delegate tasks appropriately, make informed decisions and manage all the tasks related to an Incident Response. All of the members should be trained in conducting surveys, sample collection and sample processing and capable of assisting as needed on each of the teams. It is important to realize that individuals differ in their interests, skill level, scientific biases, and endurance levels; therefore, it is important that the Incident Commander recognize their strengths and make team assignments accordingly.

The Response Team should consist of a minimum of 6 team members (1 of the 4 divers may serve as Incident Commander), with two members on each of the three teams listed below:

- **Incident Commander (IC) –** The IC is responsible for overall management of the response. This includes developing incident objectives and managing all

incident operations. The IC sets priorities and defines the ICS organization for the particular response. The IC may also be one of the members of the Survey Team, Collection Team and/or Support Team.

- **Survey Team –** The Survey Team is responsible for documenting the site (above water and underwater), collecting environmental data, mapping and delineating the affected area, documenting the affected corals and other biota, and conducting surveys. One member of the team should video and/or photo-document the scene, surrounding substrate and affected corals. Based on expertise, members can deploy transects, collect colony data, collect relevant data on affected corals, and identify corals for sampling. Their primary objective is to determine the extent of the affected area, the number and species affected (as well as those obviously not affected) and identify colonies for sample collection. It is preferable for this team to include coral biologists with at least one having some coral disease knowledge.

- **Collection Team –** The Collection Team is responsible for photo documenting colonies before and after sampling, collecting water, sediment, coral mucus and tissue-biopsies from reference tissues and lesions, recording relevant data on standardized data sheets and transporting time-sensitive samples to the surface. It is preferable to have one member serve as the bag handler and data recorder, assisting the sample collector by providing appropriate tools and pre-labeled sample bags in a sequential manner.

- **Support Team –** The Support Team is responsible for sample processing and data recording procedures that are conducted both on boat and land. This team may also shuttle samples from the collection site to the boat, and assist in coordinating other on-boat activities. It is preferable for one member to include a laboratory-trained technician capable of handling biological samples for microbiology and molecular procedures or contaminant chemistry, if indicated.

2.6.2 Training

Properly trained individuals proficient in investigative procedures, data collection, specimen collection and handling collection techniques are critical to the success of an investigation. Various formats are important to ensure properly trained teams, including lectures and field practicals, videos and web-based refresher training programs, each designed to develop and maintain essential skills. The topics should include:

- The need and purpose of conducting an outbreak investigation, and differentiating these procedures from a monitoring or research project
- Expected scenarios, and how to plan for varied situations
- Work standards, importance of following an Incident Command Structure and completing assigned tasks

- Decision making, criteria and procedural guidelines
- Procedures and techniques for collection of specimens, field surveys, and completion of data forms:
- Handling, preservation, transport and tracking samples
- Communication and follow-up
- First Aid and Safety

Proficiency Drills

Proficiency drills are exercises both 'on paper' and in the field that give the team experience in reviewing each step in the response, from initial report to closing the investigation. The drills should consist of varying scenarios that include determining each member's proficiency in conducting their assigned task, checking the condition of equipment, testing strategies for developing an action plan for a given situation, practicing collection techniques and methods as well as sample handling, processing and preservation techniques. This type of training is essential to identify deficiencies, correct a problem before an actual incident occurs requiring a coordinated response.

2.7 Logistical Considerations

2.7.1 Personnel sources

Investigative teams may be composed of individuals from local, state and federal agencies, academic institutions, non-government organizations, and trained volunteers. It is critical to maintain an updated list of contact persons and their telephone numbers, email and surface mail addresses. (See Chapter 3 for details.)

2.7.2 Equipment

General Categories

- **Boats:** It is important to identify various agencies that can respond with small vessels to support dive operations. This may include state marine resource agencies, the National Park Service NOAA Offices such as National Marine Sanctuaries, National Marine Fisheries Service, National Ocean Service, or commercial charter companies.
- **Dive gear:** Most likely responders will have their own wetsuits and dive gear; however it is important to identify local dive shops for tanks and air and other equipment as necessary.

- **Medical supplies:** Identify local hospitals, veterinary clinics or marine labs in the area as these facilities can be a valuable resource for various types of equipment and supplies, such as liquid nitrogen, preservatives, and histological fixatives. (See Chapter 4 for details)

- **Sampling Kits:** Pre-assembled kits will vary depending on the reported circumstances. Items often pre-assembled include data forms and pencils, tissue biopsy tools, markers (e.g., flagging tape, colony tags, buoys lift bags), preservatives and storage materials for samples, collection equipment (e.g., hammer, chisel, leather punch for coring or clippers for branching corals, swabs, sampling tubes, bags), dry shippers (aka: liquid nitrogen vapor shipper), coolers and cameras. (See Chapter 4 for details)

- **Freezers:** It is ideal to be prepared with your own dry shipper (i.e., cryoshipper), but in cases where this is not available, interim refrigeration resources are required. Often for frozen samples requiring lower than -20ºC, dry ice can be obtained from local grocery stories and liquid nitrogen from specialty gas suppliers. University research facilities often have -80ºC freezers that can provide temporary storage until shipping can be arranged. Hospitals often have supplies of liquid nitrogen that may provide a stop-gap in an emergency situation. It is prudent to identify venders in your region that offer either liquid nitrogen, dry ice or -80ºC freezer capability.

- **Safety:** Adherence to institutional specific dive regulations and standards must be observed and enforced. All divers should be certified, trained and proficient in appropriate dive techniques, including dive planning, proper buoyancy, bottom times and safety stops, and effective communication with designated dive buddies. The IC should maintain a log of all divers and dive profiles, and review dive plans with the designated Dive Master before and during the event. First aid and oxygen delivery kits should be on board along with the phone number of local Emergency Medical Services, hospitals and location of nearest decompression chamber and hyperbaric medical units.

2.7.3 Supply Sources

Identify sources, addresses and telephone numbers and websites of local or closest sources of supplies (see Appendix VII for suggested list) that maybe need such as:

- Biopsy corers, clippers, hammers, chisels and other hardware
- Dry ice or liquid nitrogen
- Nearest shipping address for air and ground receipt of goods and supplies
- Nearest location for shipping including airlines, courier or express shipping
- Shipping containers and necessary forms, labels and documentation for shipping

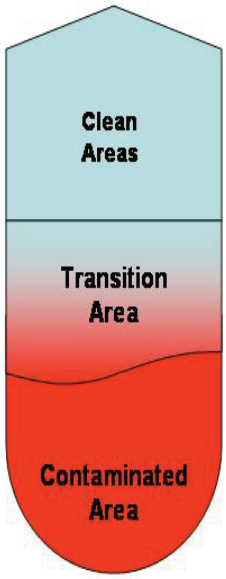
2.7.4 Lodging for Response Personnel

Identify locations for lodging response personnel. In addition to local motels, there are frequently marine laboratories with dormitories or housing available for scientists at nominal rates.

2.7.5 Work Areas and Work Flow

Identify working areas and establish a standard operating procedure for work flow, to eliminate cross contamination and attend to time-sensitive samples promptly. As part of any Incident Response there are generally three types of areas designated:

- Clean areas - Clean areas include the command post, meeting rooms, eating areas and equipment and supply receipt areas.
- Transitional areas – These areas are primarily for decontamination of personnel and equipment.
- Contaminated areas – Underwater these are areas with diseased corals. Topside or on land these areas include areas designated for sample processing or laboratory tests.



Chapter 3

Incident Command System

3.1 What is ICS?

The Incident Command System (ICS) (US National Response Team 2000a) is a standardized emergency management strategy that is part of the National Interagency Management System (NIMS). It is used in cases that require a joint effort involving multiple agencies or organizations, and provides a comprehensive framework for managing emergency and non-emergency events. Originally created by fire departments to coordinate their efforts, it has expanded to be used as a more general response network plan. This system allows response without the boundaries that can be created by jurisdiction issues. ICS decreases the duplication of efforts, and coordinates different organizations into one operational unit.

Federal directives mandate use of the Incident Command System (ICS) by their agencies. The Coral Disease and Health Consortium (CDHC) is supported by federal funds and operates as a consortium that includes NOAA, the EPA, and DOI, as well as academia, industry and NGOs, and therefore has incorporated structure and functions into their Coral Disease Outbreak Investigation Response Plan that are consistent with the ICS structure.

ICS uses distinctive titles for each organizational level. The Incident Commander oversees all responsibilities associated with a response. The Command Staff includes the Liaison Officer, Safety Officer and Public Information Officer. The General Staff consists of leaders of the Operations, Planning, Finance/Administration, and Logistics sections. Each is titled a Section Chief, and each report directly to the Incident Commander.

3.2 Adaptation of ICS to Coral Disease Outbreak Investigations

Every response, no matter the size, has an initial response phase. For a Coral Disease Outbreak Investigation, this begins with an observation by a diver of a situation they deem as unusual that involves diseased coral that is reported to the CDHC. This is, followed by notification though the Response network to the area's Regional Coordinator (Level I Report; Appendix II). A series of events are then initiated that includes: 1) evaluation of the report by experts; 2) identification of possible responses (Level II or Level III; Appendix III & IV); 3) recommendation of possible steps to reduce spread (i.e., quarantine); 4) further assessment of the situation to identify conditions that may aggravate or mitigate the event; and if warranted; 5) mobilization of a Response Team to document and characterize the incident, and collect and stabilize samples for further analyses. These steps may be moved through more quickly, if for example, an

experienced research diver familiar with disease is the original observer (e.g., a Level I report could directly trigger a Level III response).

Once a Level III Response has been declared and the Response Team deployed, the ICS structure and protocols are engaged. This process should be moved as quickly as possible from beginning to end, although in reality it may require up to several weeks to accomplish. The availability of resources (both people and materials) and safe weather conditions will influence the timeline.

3.3 Incident Command System Operational Period Plan

Steps of ICS	Steps of Coral Disease Outbreak Investigation
Incident Occurs	**Observation by someone in the field**
Notifications	**Level I Data Sheet**
Initial Response and Assessment	**Regional Coordinator Interviews Observer, and may conduct a Level II investigation to collect more detailed information. Information is discussed with National Coordinator and Expert Working Group**
Incident Briefing 201	**If the National and Regional Coordinators determine need for a Level III Response, an ICS 201 Incident Briefing ends the Initial Response Phase and launches the ICS process**
Initial Unified Command Meeting	**Use ICS 202 Form to record established jurisdictional limits, operational period to be used in the response, and agreed upon overall response objectives and priorities**
Develop Tactics/ Tactics Meeting	**Coordinators consult others as needed to determine samples to be taken and to train teams in protocols**
Develop priorities, objectives and strategies	**Coordinators determine priority for list of objectives, plan sequence of events for safest and most accurate collection**
Planning Meeting	**All Command Staff and General Staff meet to write the Incident Action Plan**
Incident Action Plan (IAP) preparation and approval	**Purpose of the IAP is to develop the response strategy for the next operational period (OP) and give specific direction to responders. The IAP only contains information needed by the responders to safely conduct the assigned action. ICS-204a form/assignment sheets**
Operations Briefing	**Incident Commander meets with all team members to cover IAP, divide responsibilities and cover safety**
Execute IAP	**Dives to collect samples and data, input and organize data, process and ship samples**
Initiate planning for the next operational period	**Debriefing of teams each night leads to modifications of IAP as needed.**
Assess progress	**Executive Summary Package can be prepared each OP to provide updated incident status report. May contain ExSum form, Situation Map, ICS-209 form, the General Plan, ICS-220 or others**
Unified Commands Objectives Meeting	**Coordinators meet to determine final steps, referrals, recommendations**

Figure 3.3 Incident Commander = Regional or Local Coordinator responsible for onsite response; IAP= Incident Action Plan

3.4 ICS Roles and Responsibilities

3.4.1 Five Management Activities of ICS

- **Incident Command Responsibilities** are to set objectives and priorities with **overall management responsibility** for the incident. Safety, Liaison, and Information functions, unless assigned to Command Staff chiefs who report directly to Incident Commander are included in the IC responsibilities.

- **Operations Responsibilities** are to conduct tactical operations to **carry out the action plan**, develop the tactical objectives and organization, and direct all resources.

- **Planning Responsibilities** are to **develop the Incident Action Plan** to accomplish the objectives, collect and evaluate information, track resources status, and document the response effort.

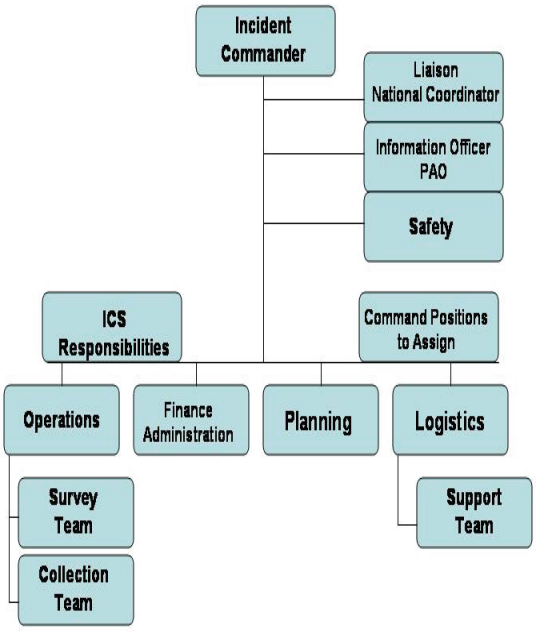


Figure 3.4.1 Overall management structure of an Incident Command Operation

- **Logistics Responsibilities** are to **provide support** to meet the incident needs, provide resources and all other services needed to support the incident response.

- **Finance/Administration Responsibilities** are to monitor costs related to the incident, provide accounting, procurement, time recording, and cost analysis.

Small incidents may be managed by one person, as the Incident Commander, in charge of all five management activities. Larger events will require that the other four management activities be assigned to command staff. Span of control is maintained at 3-7 responders per supervisor.

3.4.2 Incident Commander

The Incident Commander has ultimate responsibility for all five management activities of the IC System. In the case of coral disease outbreak investigations, it is important that the Response Team include a Divemaster responsible for the development and evaluation of the dive plan and supervision of the actual dives. Each of the Response Team members (Survey, Collection and Support Teams) reports to the IC. The IC must also ensure that the Incident Action Plan (IAP) and Site Safety Plan (SSP) are followed. The IC will develop the objectives and tactics to be included in the IAP. Other responsibilities

include keeping records of expenses to be sent to the National Coordinator, and maintenance of Incident History and Status Information (including weather and disease) records. The Incident Commander will most likely designate Planning and Logistics Chiefs to handle those responsibilities. Both of these individuals report to the IC, and the IC reports to the Regional and National Coordinators; in some cases the Regional Coordinator could also be the IC.

3.4.3 Planning Chief

The Planning Chief (PC) is responsible for collection, evaluation, dissemination, and use of information about the development of the incident and determines the status of resources. The PC is responsible for writing the Incident Action Plan (IAP). This plan includes tactics, or methods that will be used to complete the goals of the plan. Tactics should address the timing of the response, transportation of team members, and organization of samples, collection protocols, and a materials/equipment list. As the investigation progresses, the Planning Chief prepares status reports and keeps the IC informed of progress. Other responsibilities include: collecting weather information and case history (last few months- any major weather events, SST changes, new industry, overflow, etc.), obtaining necessary permits for dives and collection of samples, and making contact with laboratories to arrange for analyses of samples.

3.4.4 Logistics Chief

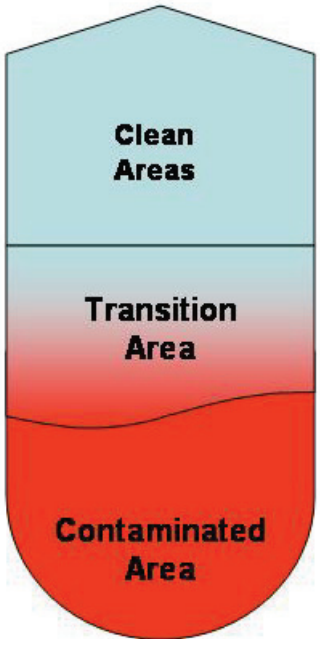
Logistics chief is responsible for:

- Facilities, services, and material support to the incident response
- Lodging accommodations for response team members
- Ordering supplies, kit materials
- Transportation arrangements for personnel, as needed
- Food arrangements, as needed
- Boat and Captain acquisition
- Evaluation of vessel operator qualifications
- Obtaining copies of training certificates and authorizations (given to the Incident Commander to be filed with the Safety Plan)
- Contacting shipping company and arranging shipment

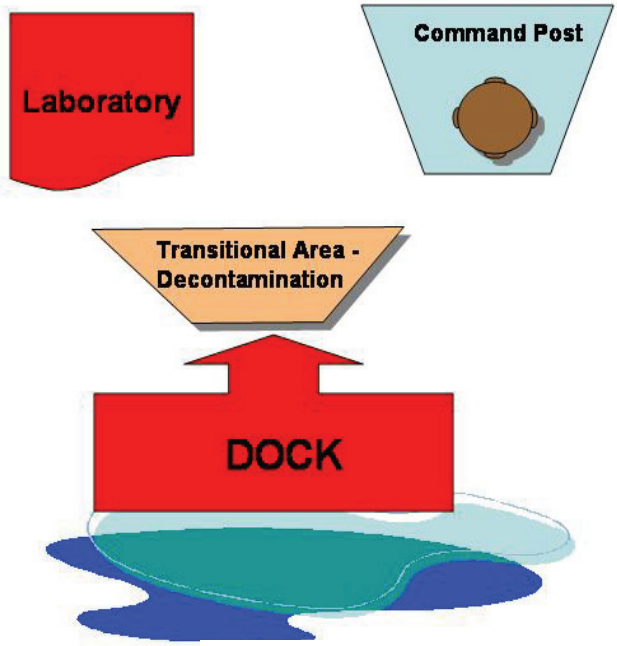
**Liaison Responsibilities*
The National Coordinator will serve as the Liaison Officer during a response. The Liaison Officer is responsible for addressing the concern of local agencies affected by the incident and communicating that information to the Incident Commander. The LO should also identify stakeholders and address their concerns.

3.5 Establishing a Command/Operations Center

The Operations Center is established to continually monitor any incoming information regarding the response, either by phone, fax or email, and to communicate with Coordinators as well as federal and local authorities and involved organizations. This may be a research center, university office, local or federal government office (i.e., National Marine Sanctuary, National Park Service), or similar location, to be determined by the Incident Commander. Promotion and public awareness of the response may also be run from this office, or that of the designated Public Affairs Official. This location also serves to coordinate the response, check in and deploy team members, gather and archive data, and keep track of samples and their shipment/receipt.



Boat Situation



Land Situation

3.6 A Model Response & Decision Making Process

3.6.1 Notification: Level I

A model response begins with an unusual observation in the field. This observation is most easily verified if the observer is a trained coral reef scientist, but it could come from anyone who describes specific coral conditions indicative of disease. The importance of outreach is highlighted at this crucial first step, as we need to make the reporting format readily available to those making the observations. The reporting form will be available on the CDHC website and also available at local sources such as dive shops. This Level I (Appendix II) information is completed and sent to a Regional Coordinator. The observer may be a recreational diver, dive operator, manager or researcher who observes an unusual disease outbreak and notifies the Regional Coordinator directly. In this situation, the Regional Coordinator could fill out the Level I response form during the interview.

The Regional Coordinator (Appendix I, Regional Coordinator Check List) will contact the Initial Observer to conduct an interview and:

- verify the report
- determine the validity of the report
- collect sufficient information to determine the need for further response
- request additional supporting materials, if available, such as photographs

At this point, the Regional Coordinator may elect to close the case and forward the report to the National Coordinator. If this is a new observation, a species at risk, or if a large magnitude incident is suggested, the Regional Coordinator may recommend a Level II response, notify the National Coordinator, and begin to organize a small-scale trip to the site to collect more specific information to determine whether the incident may warrant a Level III investigation. The Decision Tree below illustrates (Figure 3.6.1) this process.

Coral Disease Investigation Decision Tree

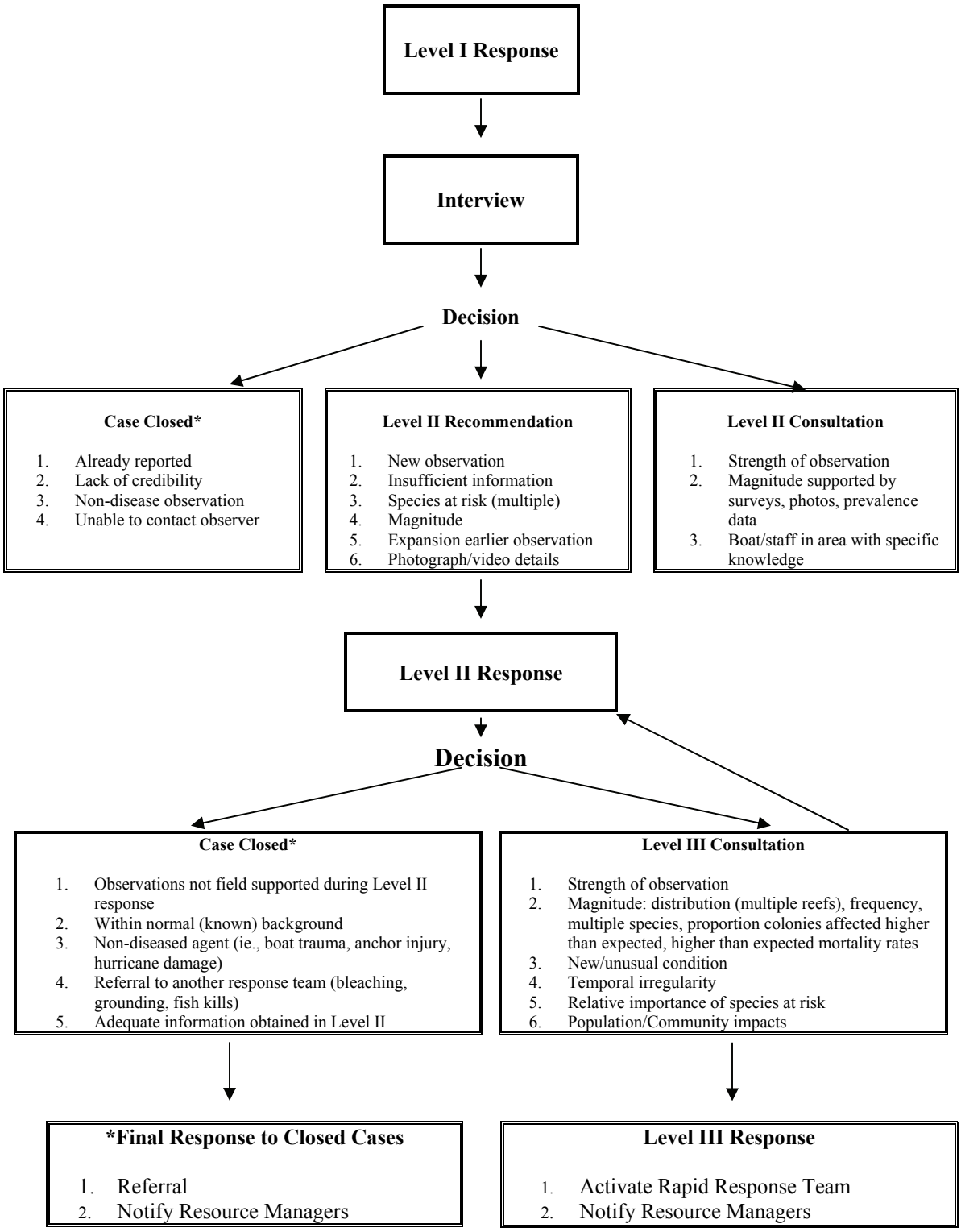


Figure 3.6.1 Decision Tree for Outbreak Investigations

3.6.2 Disease Determinations: Level II Decisions

3.6.2.1 Disease vs. Damage

In corals, gross morphological signs of disease can be manifested as loss of tissue, damage to the underlying skeleton, alterations in color of affected tissue, or changes in shape, size, or texture of the corallum (Work and Aeby 2006). These signs alone can only be used to describe an observed lesion, not to infer causality. Additional morphological features (e.g., histology) and laboratory studies (molecular, microbiology, analytical chemistry) are needed to better characterize the etiology, and determine possible causes. Many lesions found on corals are not the result of an infectious agent but rather a result of physical damage (e.g., predation, abrasion, and storm damage), environmental stressors (e.g., temperature-related bleaching, sediment stress), or chemical damage (e.g., allographic competition, or toxicants). When a coral lesion is encountered in the field it is important to conduct a thorough scene investigation to eliminate predation, bleaching, storm damage, competition and overgrowth, algal interactions and other potential sources of mortality. Some investigators use a small magnifying hand-held lens to help discern these characters underwater, if features are still questionable, a portable dissecting scope, on the support vessel can help in determining more specific features of the lesion. If evidence suggests the lesion may be due to disease, and lesions are widespread and are causing extensive mortality or atypically affecting a certain species, recommendations should be made for an investigation with sample collection for laboratory analyses.

3.6.2.2 Characteristics to Consider for Disease Determination

- **Growth anomalies**

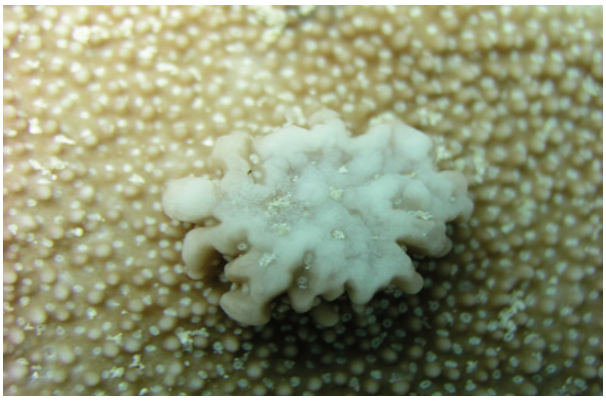


Figure 3.6.2.2.1 Coral growth anomaly *Photo courtesy of Andy Bruckner, NOAA*

Although frequently referred to as 'tumors', the abnormal growths found on more than 40 species of coral, result from several different growth processes, most of which do not fit the strict pathology for tumor definition, i.e., neoplasm (uncontrolled growth of abnormal cells). Frequently the anomalous growth forms are due to processes such as hypertrophy or hyperplasia (Domart-Coulon et al. 2006). In general, the growth anomalies of coral are focal or multifocal, circular to irregularly 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 reduced in number or 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. Aberrant calyx formation, enlarged calices, reduced number of calices, and color changes are features that may also be associated with coral growth anomalies.

- **Tissue loss**

Figure 3.6.2.2.2 Parrotfish spot biting on star coral (*Montastraea annularis*), *Photo courtesy of Andy Bruckner, NOAA.*

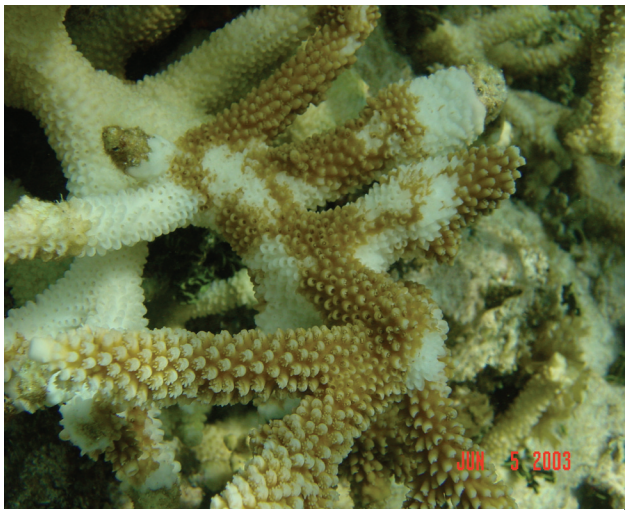


Figure 3.6.2.2.3 Acropora disease of unknown etiology (2003 case from FL Keys), *Photo courtesy of Dana Williams, NOAA*

It is easy to jump to conclusions when faced with corals having complete tissue loss. Caution should be exercised not to assume the lesions are a result of an infectious agent. Lesions can be caused from a variety of factors that include physical damage, environmental changes (e.g., temperature), toxicants or infectious agents. Therefore it is imperative that lesions be inspected closely for distinguishing signs. Knowledge of local predators and their associated feeding patterns is crucial in distinguishing predation from disease. Predators will not necessarily be on the affected coral colony, but their presence in the vicinity should be noted, e.g., snails often can be found hiding at the base of the colony or in other crevices nearby. Many predators are known to cause complete tissue loss. Crown-of-thorns starfish (COTs) often leave a 'trail' of damaged colonies which can lead to the culprit. Coral-feeding fish, on the other hand are usually within the vicinity, as they are site attached. With tissue loss it important to note the pattern of tissue loss, rate of tissue loss, and presence of loose tissue. Presence of loose tissue can suggest a "sloughing" that occurs during some infections. Another sign of disease is progressive tissue loss. The rate of tissue loss can often be estimated by the degree of algal colonization on the bare coral skeleton. Frequently, disease will produce a linear pattern of progressive tissue loss as opposed to a more amorphous pattern associated with certain predators.

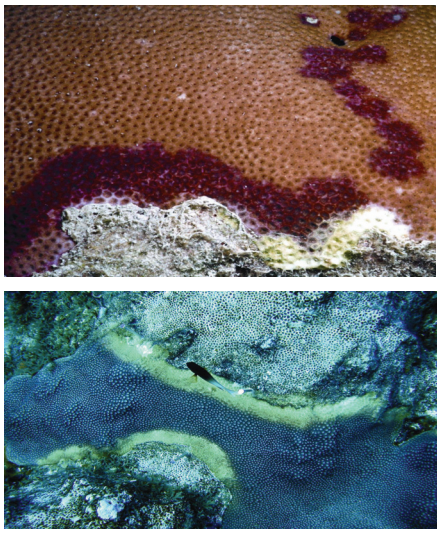


Figure 3.6.2.2.4 Above *Siderastrea siderea* with Dark Spots Disease with some associated tissue loss (white skeleton) in the lower portion of the photo, below Yellow Band Disease on *Montastraea faveolata*. The colony exhibits minimal recent tissue loss. *Photo courtesy of Andy Bruckner, NOAA*

- **Color change**

If there is color change, the observer should look for evidence of interactions with other organisms (coral, algae, other invertebrates). If the colony is white, examine the skeleton and differentiate between bleaching, where the polyps are still present, and tissue loss with a bare skeleton.

Field diagnosis is only the first step in determining presence of coral disease. If the signs of disease are present, etiologic diagnosis will require histological and other laboratory analyses. Level III investigations should be recommended only when the accumulated facts in the case meet the criteria for an unusual disease occurrence (see section 3.6.3).

3.6.3 Decision to Launch an Investigation: Level III

Level II disease assessment and information is forwarded to the National Coordinator and the Expert Working Group (EWG) for consideration. The criteria for determining whether reports constitute an Unusual Coral Disease Outbreak in most instances include a mass mortality event and/or numerous coral colonies with gross signs of recent partial tissue loss and they meet one or more of the following criteria:

- Species of interest is affected (e.g., ESA-listed coral)
- Multiple species affected
- Disease appears at an abnormal time or place
- Frequency of disease (i.e., increased number of colonies affected with lesions) is greater than expected for that time of year or for that location
- Potential ecological effects of concern
- Signs consistent with a known or reported disease, but affected species previously thought to be resistant to that disease
- Possibility of new disease, i.e., disease signs are not consistent with previously described clinical signs

The EWG and the National Coordinator, in consultation with the Regional Coordinator will use their knowledge, experience and judgment to determine whether any of the criteria apply in a way that warrants an organized investigation with sample and data collection, or Level III Response.

3.6.4 Launching an Investigation: Level III ICS

Once a Level III Response is initiated, the Incident Command Structure is implemented. An Incident Commander is appointed, and other General Staff are assigned as necessary. The Incident Commander is responsible for collection of a Case History, Development of an Incident Action Plan (IAP) and Site Safety Plan (SSP), organization of all response team members, acquisition of needed equipment, permits and sampling kits, and execution of the IAP including sample collection, processing and shipment.

A Command Post is designated, where all team members can report and be briefed on the IAP and SSP. Once the field investigation begins, Survey Teams conduct the initial dives to collect site data, set the perimeter of the affected area, document the scene, deploy and assess transects, and identify colonies for sample collection. The Collection Team obtains samples of coral tissue and mucus and associated water and sediment. The Support Team aides in these dives, processes or stabilizes the samples once they arrive on the boat, and finalizes the processing back at the Field Lab. All samples are stabilized, properly processed according to the planned laboratory analyses, logged, labeled, and shipped to the appropriate diagnostic laboratories. The Incident Commander calls to verify safe arrival of samples to the appropriate contact person at each laboratory. Any media information is supplied by the appointed Public Affairs Official for the region. Throughout the Outbreak Investigation, a cycle of planning, execution, reassessment and adjustment to plans will assure the most effective action (See Response Cycle, Figure 3.6.4). Debriefing at the end of each response day will help to identify areas for improvement in the next dive or next day's tactics.

Once the field investigation portion of the response is complete, and feedback is given to the Initial Observer and other management authorities involved in the response in the form of a Quick Report, which is an update that includes any preliminary findings and a summary of laboratory tests being conducted and any follow-up observations recommended. Recommendations are formulated by the Incident Commander, Regional Coordinator and/or National Coordinator and provided to all of the appropriate parties. Analysis reports from samples sent to designated laboratories are collated in the National Office and reviewed by the Expert Working Group and other medical experts as needed, to evaluate the test results and provide a diagnostic report. For example histopathology samples are archived at the International Registry of Coral Pathology at the NOAA Cooperative Oxford Laboratory, Oxford, MD and microbial pathogen screening tests may be conducted at NOAA NOS CCEHBR, Charleston, SC. All information associated with the Response is kept on file by both the regional coordinators and national office, for future reference.

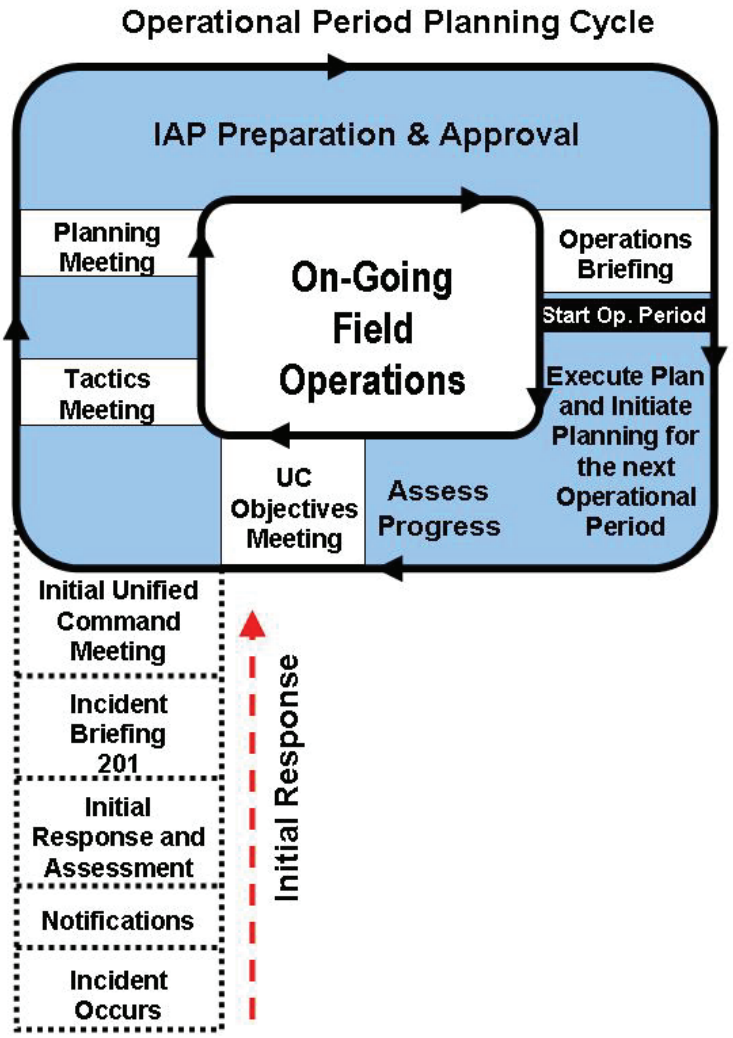


Figure 3.6.4 Flow diagram of operations during a Level III response. *Artwork by Thomas Bartlett.*

Chapter 4

Case History, Sample Collection, Processing and Shipment

4.1 Case History

A case history is a chronological record of significant events and observations surrounding an outbreak of disease and should be the first entry in a case file for a given outbreak investigation. Detailed field observations during an outbreak and investigation that identify significant events that preceded the outbreak can provide valuable context for interpreting the analytical data. Perceptive, thorough observers are invaluable for an investigation and avoiding preconceptions are imperative if the investigation is to remain unbiased.

Environmental Factors

Environmental changes such as storms, heavy rains, abnormal temperature shifts, changes in water quality can be sources of stress that contribute to outbreaks or mass coral bleaching events. Satellite imagery over the previous months may reveal unusual situations such as large run-off or storm water inputs and should be included as data and information are gathered. It is also important to determine if there were recent industrial or agricultural spills or applications of pesticides or herbicides in the vicinity. Any previous disease outbreaks or die-offs in the area should also be noted.

Estimate Onset of Disease

The timing of disease onset and rate of progression can be estimated for some corals by the degree of bleached tissue or bare skeleton is associated with the lesion and the amount of algal colonization. Note and photo-document algal colonization of affected colonies.

Species and Number Affected

It is important to document which species are affected as well as those that remain unaffected as some diseases appear to infect only a narrow host range while others a much broader range. The incidence or proportion of sick colonies, verses the number of those not displaying lesions is also valuable to the diagnosis.

4.2 Basic Steps

General Considerations

Field observations and data provide a critical link in disease diagnostic work and can significantly affect the outcome of laboratory efforts which depend on the quality of the

samples and the accuracy of the accompanying observations and measurements. The quality of the information depends on a number of factors which include:

- Response Team size, skill and experience
- Response Team organization, interests and biases
- Adherence to clear, detailed protocols, including time-sensitive samples
- Adequacy of planning and suitability of logistics (e.g., type of boat)
- Conducive weather conditions to support safe dive operations
- Care maintained in labeling, processing, stabilizing samples
- Care in adhering to shipping and storage guidelines

4.3 QA/QC Considerations

Minimizing Cross Contamination:

- Visit sites with no signs of disease first
- Sample healthy coral first, then affected/diseased coral
- Use disposable nitrile gloves that are changed between each colony visited
- Gloves will be placed in a 'trash' container underwater and on the surface placed in a second plastic bag where they can be disinfected with bleach and then disposed with garbage
- Use new or decontaminated equipment for each sample.
- On the boat, decontaminate collection equipment by soaking in dilute hypochlorite (5-10% bleach) solution for at least 10 minutes and rinsing in fresh water.

Decontaminating Dive Gear

- Clean dive gear by soaking in decontaminating solution
- Rinse thoroughly in fresh water at the end of each dive.

***Laboratory experiments have been conducted to determine cleaning agents that are effective in disinfection, yet pose little threat to dive gear deterioration. The suggested agent to date is 5% bleach prepared fresh or 3% Lysol™ (diluted according to sanitization strength on packaging and followed by a thorough fresh water rinse).*

4.4 Survey Team- Site Identification and Assessment

The Survey Team will consist of 2-3 members. The primary responsibility of this team is to describe the scene underwater. This includes defining the perimeter of the outbreak, describing the biota, including cover, diversity and presence of other stressors, describing the extent of the outbreak (i.e., determining prevalence: number of species and individuals affected in the context of unaffected individuals), characterizing the lesions, and marking colonies for collection.

4.4.1 Duties of Survey Team

To accomplish the initial site assessment all available divers* should be used to:

- Conduct a survey of the area, (if available, with tow boarding or underwater scooters) to determine the spatial extent of the outbreak

- Count colonies in duplicate (once by each partner) and record the condition of corals within replicate belt transects. Optimally a minimum of two 20 x 1 meter linear transects within the center of the affected area and two transects at the perimeter should be completed (where possible). The dimensions of these transects may vary depending on the size of the affected area, the abundance, diversity and cover of stony corals, depth of the affected area, and size of the team; it is important to capture the diversity of a particular area within chosen levels of statistical confidence. **Note that some areas are more conducive to belt transects and may be the preferred methodology.*

- Characterize affected colonies within a 20 x 1 meter belt (colony size, severity of lesion, genus or species). The level of detail (e.g., type of colony measurements) depends on the available time, size of response team, and level of expertise of the team.

- Assess cover of corals and other major biota

- Record all information on Survey Data Forms

****if numbers of dives per person are restricted, this may be limited to Survey Team members***

4.4.2 Individual Operations within the Survey Team and Their Responsibilities:

- **Videographer-** Video document the site from both planar aspects (at one depth along transect, keeping camera a set distance from the substrate as appropriate for the visibility, relief, cover and size distribution of corals) and pan video to get documentation of general habitat.

- **Cartographer-** Create a generalized map of the area with key landmarks and GPS coordinates noted to allow orientation and ability to triangulate to specific colonies upon follow up visits.

- **Tactical Specialist-** Identify affected individuals and temporarily mark them for sampling. The use of temporary floating chains (plastic chains) to mark colonies is suggested. Record Global Positioning System (GPS) location, depth, and other data designated on the Sample Site Documentation Form. Assign a unique identifier and photo-document each colony marked for sampling.

4.4.3 Survey Approach

Collection of epizootiological data should include, at minimum, the spatial extent of the outbreak; magnitude in terms of the number of colonies affected; and severity, in terms of the percent coral tissue affected or mortality resulting from the outbreak. A standardized disease response should include the following:

- **Broad surveys** to characterize the habitats affected, spatial distribution (e.g, habitats and depths affected) of affliction, and a rapid assessment of potential physico-chemical parameters (depth, water clarity, temperature, nutrient load, etc.), and anthropogenic impacts (pollution, runoff, sedimentation, etc.) that may be linked to the outbreak;

- **Characterization of community structure** in terms of cover of major benthic attributes (substrate, algal abundance and type, and coverage of benthic invertebrates by major phyla or class);

- **Population information** of the scleractinian corals (i.e., abundances, size classes, species diversity, and health status); and

- **Detailed disease assessments** including quantification of susceptible species and the diagnostic features of lesions on individual affected corals including photographic records of the lesions.

The primary survey approaches are described below:

- **MANTA TOW SURVEY:** The method can be used to estimate coral cover, dominant coral types, and broad patterns of disease or mortality. It is not possible to collect detailed diagnostic or quantitative data using this approach.

  A snorkeler (or diver) is towed over the reef by a small outboard motor boat to characterize the major habitats, reef zones, major structural attributes, percent cover of major groups (e.g., stony coral, algae, soft coral, hard bottom), and spatial extent of the disease outbreak, noting areas with the highest prevalence. The snorkeler can drop marker buoys to delineate the area affected by disease. The precision of the manta tow surveys is limited by visibility and depth of the

site, complexity of the reef, and expertise of the observer. One advantage of the technique is that it enables the observer to characterize representative habitats in the context of the entire reef environment.

- **POINT INTERCEPT SURVEY:** Biotic and abiotic components are recorded at certain pre-defined intervals along transects to collect information on cover of various benthic organisms including coral as well as substrate types.

Diver one extends a transect 20 m, parallel to depth gradients, within the approximate center of the affected area. The diver then slowly swims back to the beginning of the line recording the substrate type, and/or organisms to the highest taxonomic resolution possible under the tape every 0.5 m (total of 40 points per line; at minimum, 2 transects should be completed within the outbreak area and two outside of the main affected area). The cover of each component is then determined by dividing the number of points containing the specific category by the total number of points examined (and multiplying by 100). The minimum type of data collected for each point should include:

  - Substrate type: recorded as hard bottom, rubble, sand or dead coral
  - Specific type of algae or invertebrate to highest taxonomic resolution possible. The categories can include:
    - Algal assemblage: recorded as fleshy macroalgae, turf algae, erect coralline algae (e.g., *Halimeda*), crustose coralline algae, and cyanobacteria
    - Stony coral, recorded at minimum to genus
    - Other invertebrate, including sponge, soft coral, gorgonian, anemone, bryozoan, tunicate etc. These organisms should be recorded by major group, and if possible, also include growth form and taxa to highest level possible.

- **CORAL ASSESSMENT SURVEY:** All corals within a predefined area (i.e., 1 x 20 m) are counted and measured and the presence of disease is recorded. This approach will provide detailed data on disease prevalence based on a whole colony assessment, population dynamics, and health status.

Diver two records all colonies (species, maximum diameter, and condition) within one meter of the transect. A 1 m bar marked in 5 cm increments is used to help guide estimation of transect width and to guide estimation of colony size. Only colonies with whose centers lie within the belt transect are recorded; large colonies with their centers (e.g., more than 50% of the colony) lying outside the transect must be ignored.

Colony sizes are preferentially recorded to the nearest 5 cm from a planar view, with measurements only of corals 10 cm or larger in diameter. If the site contains a very large number of colonies, size classes can be lumped into six groups: 10-20 cm; 21-40 cm; 41-80 cm; 81-160 cm; 161-320 cm; and >320 cm. Smaller

colonies should be identified (at least to genus) and counted within the 20 X 1 m belt, lumping them into colonies 0-5 cm and 6-9 cm. If there are large numbers of small colonies, these can be quantified by recording the total number within five 1 m^2 quadrats per transect instead of surveying the entire belt. Quadrats are placed next to the transect tape at predetermined intervals (e.g., 0, 5, 10, 15, 20 m). **Note for certain areas of the Caribbean, colony sizes of 4 cm or greater may need to be included in the assessment.*

- **DISEASE ASSESSMENT:** All colonies with disease or other causes of mortality are identified and counted, and specific detailed diagnostic information is collected for those corals exhibiting signs of the disease under investigation. This approach will provide data on prevalence of all diseases as well as useful diagnostic descriptions for the disease of interest that can assist in determining when the event first occurred, how severe it is, whether it is ongoing, and if it is increasing or declining in severity.

  Diver three identifies every colony within the one meter belt with signs of recent mortality, recording the genus and the common name of the disease or other condition. This includes signs of predation (differentiated into gastropod, fireworm, COTS, or fish bites), disease, bleaching, or compromised health (e.g., algal or invertebrate competition, physical damage etc.). For colonies exhibiting signs of the disease under investigation, the observer should record the genus (or species), maximum diameter, and diagnostic features of the lesion (see section 4.4.3 and Appendix IV). **If time permits accuracy may be improved by having the same diver(s) conduct the community structure surveys as well as the disease assessments.*

  This same diver also identifies corals for sampling and marks them with floating chains and assigns temporary numbered tags. Colonies for sampling should include representatives from all species affected by the disease of interest, as well as different stages in the progression of the disease ranging along a continuum from colonies that appear to be newly infected (small lesions that lack algal colonization) to older well established infections (prominent large lesions with a gradation of algal colonization on exposed skeletal surface).

Depending on the site characteristics, species diversity and abundance, and extent of the disease outbreak, coral assessments or disease assessments may take additional time to complete. As divers finish a task, they can assist the other divers by conducting coral assessment or disease assessment, beginning at the end of the transect and working towards the other divers. If the survey team consists of two divers, one diver would complete point intercept surveys and then begin disease assessment surveys, as the coral assessment may require the most time.

4.4.3.2 Possible Modifications to Consider

Many Indo-Pacific reefs are characterized by high coral cover, a large number of species and colonies, and a dominance of small to intermediate sized corals, making it impractical to measure the size of every coral, especially if dive time is limited and the Response Team is small. It is best to record corals to species, but this may be impractical or not possible on certain high diversity Indo-Pacific reefs and depending on the expertise of the survey team. In this case, divers should record corals to the level of Genus, attempting to differentiate between growth forms when possible (e.g., massive vs. branching *Porites*). The methods described above could be further modified, based on the complexity and size of the affected area, size of the team, and available time.

The minimal survey information that should be collected is an accurate list of all of the genera (or species) and their abundance within the sampling area, along with the numbers of each taxon exhibiting signs of the condition being investigated. A simple data sheet listing all the genera in the first column, a second column to tally the number of healthy corals, and subsequent columns to tally the number of colonies with each type of disease, predation or compromised health. This will provide information on the prevalence of colonies by genus (or species) that are diseased, as well as the prevalence of a particular disease for the entire coral community.

A second level of information could include recording each genera observed within the belt transect, and the numbers of each genera that shows signs of the condition under investigation. The observer could record the maximum diameter of colonies, focusing on measuring only those taxa identified as being susceptible to the particular disease.

A third level of information could involve recording the total numbers of each genera (with and without disease) within particular size classes (e.g., lump all colonies into six categories, <10 cm, 10-19 cm, 20-49 cm, 50-74 cm, 75-100 cm and >100 cm). This could be done for all genera, for a subset of the 12-15 dominant genera, or only for the genera affected by the disease.

4.4.4 Diagnostic Descriptions of Lesions from Gross Observations

For each colony exhibiting signs of the disease under consideration within the survey area, information should be recorded on the affected taxa, its size, and condition (see Appendix IV for assessment form). The lesion should be described in terms of its gross characteristics (tissue loss, skeletal damage, color change, or growth anomaly), the location, lesion pattern, lesion margin, and lesion color (See Fig. 4.4.4), including:

- Location: apical, medial or basal
- Lesion pattern or distribution: linear, annular, focal, multifocal, coalescing, or diffuse
- Lesion size: maximum dimensions

- Lesion margin: condition of disease margin. This should include the thickness, lesion shape (linear, annular or diffuse), and border (smooth, jagged, tissue sloughing).
- Rate of Progression: extent of recent tissue loss and degree of algal colonization, classified as acute, sub-acute or chronic. Colonies exhibiting rapid (acute) disease progress have prominent exposed white skeletal areas with no or minimal algal colonization by turf algae. Moderate (sub-acute) lesions are characterized by large patches of exposed white skeleton along with initial signs of turf and macroalgal colonization on older tissue-denuded skeletal surfaces. Chronic lesions often have a narrow (<1 cm) border of white exposed skeleton adjacent to living tissue, or an absence of recently exposed skeleton; previously denuded skeletal areas are colonized to various degree by turf, macroalgae and crustose corallines.

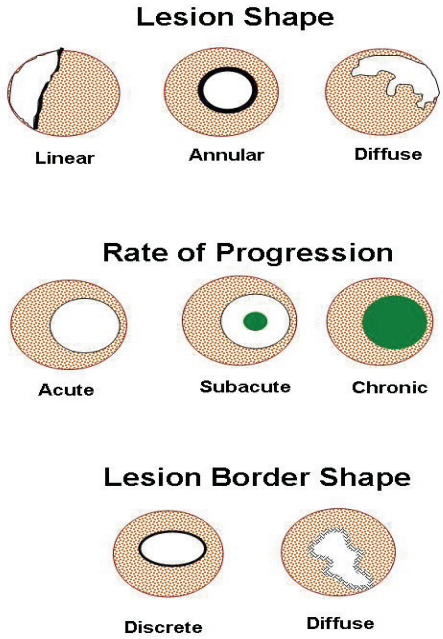


Figure 4.4.4 Diagnostic descriptors for lesions on stony corals. *Modified from (Work and Aeby 2006).*

4.4.5 Field Microscopy

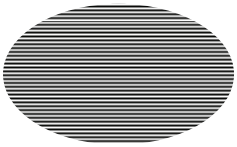


Figure 4.4.5.1.1 Resolution is the shortest distance between objects that allow each to be seen.

4.4.5.1 Introduction

Magnification is the ability to visibly scale up specimens to be able to see more detail than with the naked eye alone. It can be accomplished using a variety of techniques which increases *resolution* which is the smallest distance between two objects at which they can just be seen as two separate and distinct objects.

Magnification of specimens in the field can be accomplished by using simple hand lenses (e.g., 5X magnification) while diving or at the surface or using a simple dissecting microscopes (also called stereomicroscopes) on the boat. Using these simple tools to provide a closer look at field specimens, lesions and disease margins can provide valuable visual details that are not apparent to the naked eye and contribute significantly to the diagnostic process. For example, when observing a brown banding pattern on a coral, it is relatively easy to distinguish between tissue discoloration and a band of ciliates, when 5X magnification is used, whereas visual inspection with the naked eye alone can lead to an erroneous conclusion of tissue discoloration (Figure 4.4.5.2).

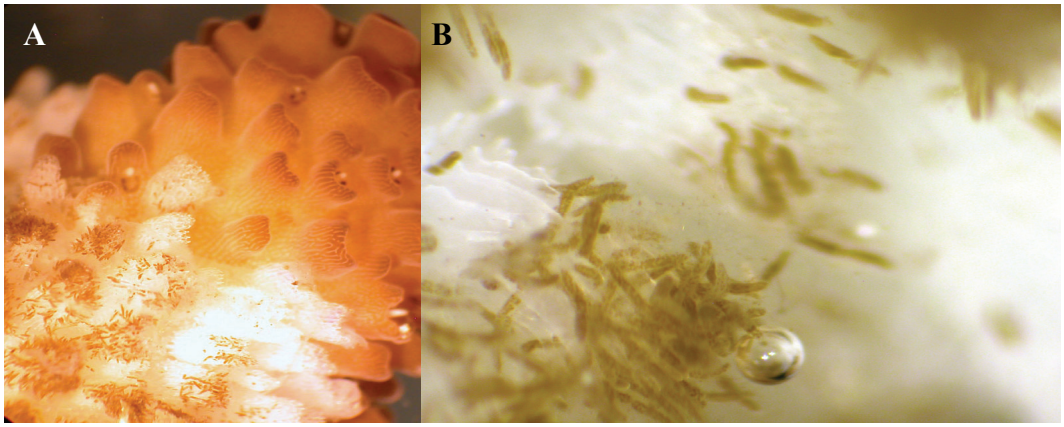


Figure 4.4.5.1.2 *Acropora surculosa*, infected by the Brown band ciliate in a laboratory aquarium tank. A= low power stereoscope B= high power stereoscope *Photo courtesy of Dr. Laurie Raymundo, Univ of Guam.*

4.4.5.2 Relative Sizes

Although visual inspections are imperative during field investigations, closer examinations with a magnifying lens or stereomicroscope is important to consider as a regular part of lesion characterization in order to see its unique physical characteristics (size, shape, motility, color). It is also important to recognize the relative sizes of cells or organisms and to put them in proper perspective and to avoid erroneous descriptions. For example, bacteria are too small to be observed with simple magnifying lenses or even a stereomicroscope since neither instrument has the ability to resolve objects in this size class. Figure 4.4.5.2 illustrates the relative sizes of specimens related to coral reefs and the instruments required to view them.

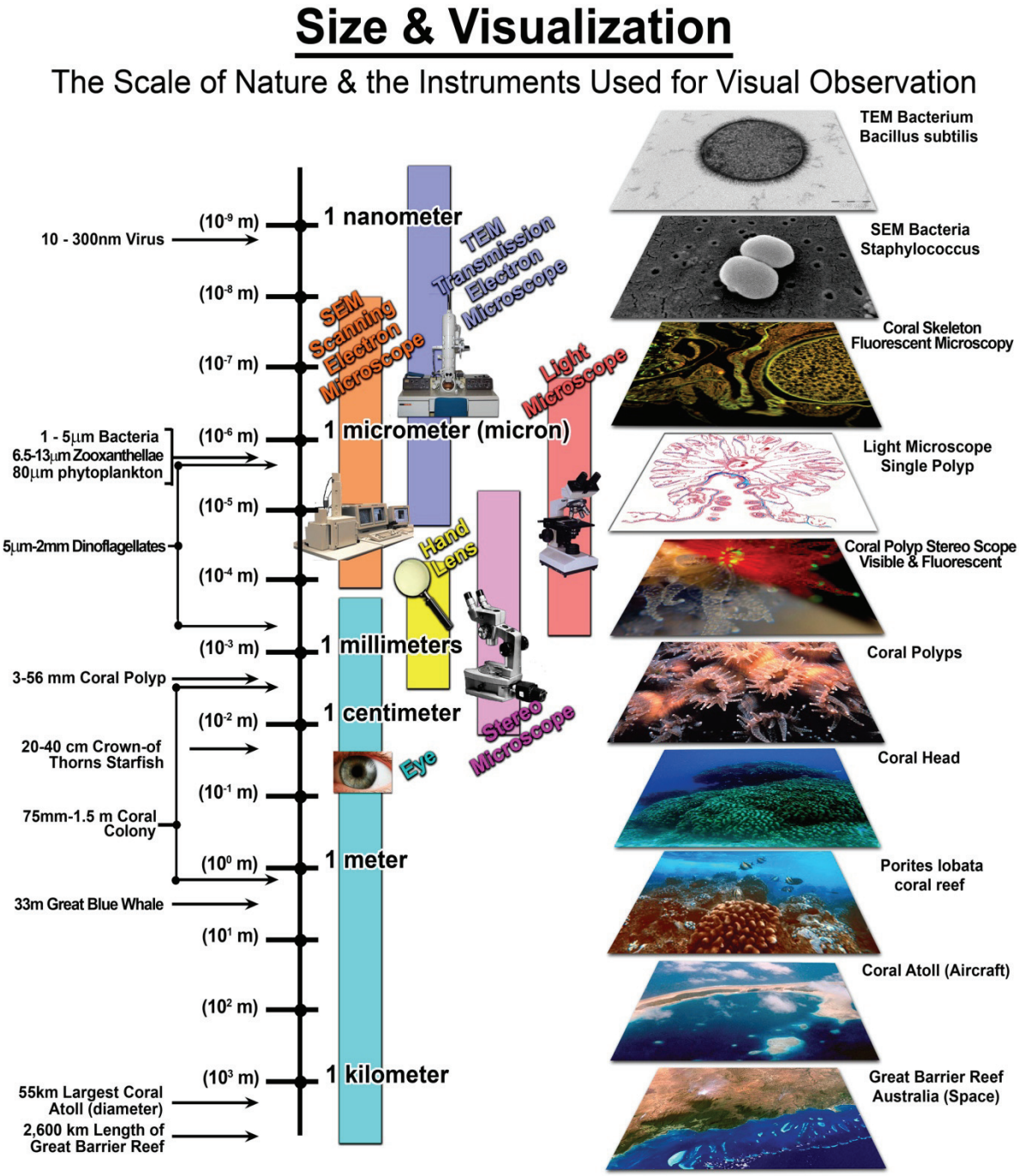


Figure 4.4.5.2 The scale of coral reefs and the instruments used to view them.
Artwork by James Nicholson

4.4.5.3 Use of Stereo Microscope

The American Optical Cycloptic microscope (Figure 4.4.5.3) is a sturdy, easy to use microscope that is available for field investigations.

- To FOCUS the microscope, use the focus knob starting above the good focus level and rack down until the specimen is in sharp focus. This particular microscope is collimated (parallel beams of light) to assure parfocality (stays in focus when magnification is changed) at all magnifications.

- To CORRECT for individual eye differences, first focus the microscope with the right eye. Turn the left eyepiece focusing sleeve counterclockwise until the left image is out of focus, then turn clockwise until the image is in sharp focus with the left eye.

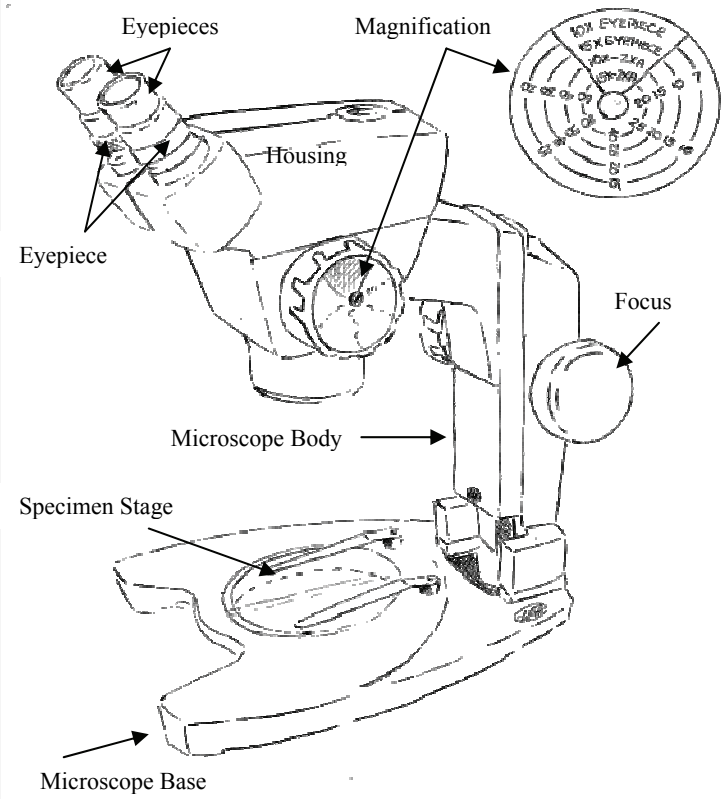


Figure 4.4.5.3 Illustration of an American Optical dissecting scope. *Illustration by Athena Avadanei*

- To adjust INTER-PUPILLARY DISTANCE, grasp the eyepiece housing and adjust the spacing by moving the two until able to view a full single field with both eyes.

- To Change MAGNIFICATION, rotate magnification knob to desired magnification. Magnification values can be read at the indicating dot.

4.4.5.4 Care and Maintenance of a Stereo Microscope

A well-designed stereo microscope requires surprisingly little maintenance. Most problems can be prevented by some simple, common sense, proactive preventative steps. Bear in mind that cleaning optics is inherently destructive over a long period of time so preventing optical contamination is better than cleaning it off. One of the most useful microscope accessories, often unused is the simple dust cover. A microscope should always be covered when not in use. Special consideration should be given to the type of cover where ever there is the possibility of water, chemicals or blowing sand affecting the scope.

Common dust is usually not of concern and if excessive enough to be bothersome is easily removed with a source of air, either commercial canned air, or an ear syringe. The most common type of contamination that requires prompt and thorough cleaning is finger prints. The oils in a finger print can actually etch the optical coatings on the lens. Eye makeup such as mascara can be a chronic problem in the contamination of the eyepieces. The best solution is to discourage the use of eye makeup by personnel using microscopes. Salt spray needs to be removed by the careful use of fresh water cleaning using damp clothes, never liquids that could get into the scope.

Tips for proper cleaning of optics:

1. Have proper materials on hand including good quality lens paper, a source of air and lens cleaner.

2. Always first use air to blow off the optical surface to remove any grit that could scratch the optics during cleaning.

3. Never touch an optical surface with any dry material. Always moisten the cleaning cloth or tissue with lens cleaner or use your breath to fog the lens.

4. Suitable cleaning materials include lens tissue, microcloth, or a well laundered clean handkerchief.

5. Clean in a circular motion without applying excessive force. Make several passes using a clean surface each time.

6. The use of solvents should be carefully restricted to lens contamination such as oil or mounting media that actually requires it. Never apply any solvent directly to a lens but always apply it to lens paper or a cotton swab. Shake off excess liquid before applying to the lens. Materials like oil will require the use of multiple swabs or papers as they must be discarded after each pass. Check all safety instructions for any solvent and make sure you have adequate ventilation, and personal protection as required.

4.5 Collection Team- Sample Collection

The Collection Team may consist of 3 members: the Sampler, Sample Handler, and the Records Diver. However, the tasks can be accomplished by 2 divers, with assistance from a snorkeler on the surface to ferry samples to the boat between each colony. The **Sampler** will physically collect the prescribed samples from coral colonies and photograph the pre- and post- biopsy condition of the affected areas. The **Sample Handler** will assist the sampler in keeping track of the collection bags or tubes, verifying the labeling, securing the samples once taken, and seeing that time intervals are maintained for time-critical samples such that the samples are transported to the surface in the prescribed time. The **Records Diver** is responsible for the Diseased Colony

Collection Form, and will ferry time-sensitive samples to the surface. The Sample Handler may also perform the duties of the Records Diver on the occasion of a two-person collection team.

4.5.1 General Considerations

Specimens are material representative of the problem and suitable for further laboratory analyses. The specimen may be tissues, mucus, environmental samples (e.g., water or sediment) or other flora or fauna that associate with the diseased corals. Photographing lesions and surrounding area provides a record of color, location and appearance of lesions. Both actual size and macro shots should be taken before and after removal of tissue biopsies. It is also important to include a color scale and metric to size and color correct photos.

The primary consideration when collecting diseased tissue is personal safety; universal precautions for potential health hazards should be observed. To avoid transmission of possible disease agents, disposable gloves should be used, and disinfection with a commercial disinfectant or a 5-10% bleach solution (prepared w/in 12 hrs of use and kept out of direct sunlight) should be used to decontaminate collection tools, work areas and dive gear. Observations of these guidelines will minimize transmission and protect team members.

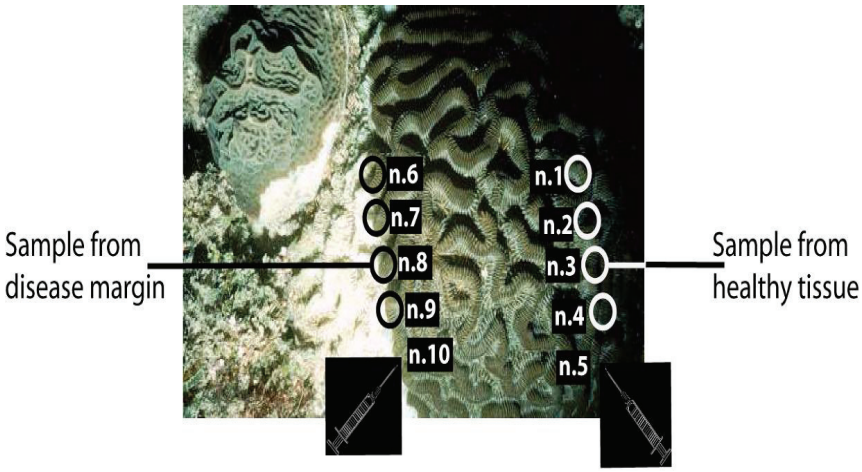


Figure 4.6.1.1 Diagram illustrating disease margin, unaffected areas and possible sampling design. *Illustration by Shawn W. Polson.(Polson et al. 2006).*

"Collection and analysis of samples is the basis of investigation, and the validity of the results and conclusions of any study is totally dependent on the quality of the samples collected" (Wobeser 1994). Properly trained individuals proficient in collection techniques are critical to the proper collection and preservation of samples. Improperly collected or preserved specimens can look the same as a good sample, but if handled improperly or contaminated, it will preclude further analyses and compromise the integrity of an investigation.

4.6 Collection Protocols for Biological Analyses

4.6.1 Labeling Scheme Guideline

- Letter or number designation of the collection site
- Four letter abbreviation for coral species (first letter of genus, first three of species)
- Colony number within site
- Two letter sample type abbreviation

Colony Type	Analyses/Collection Method		Example
Reference	**W**ater	**P**rotein	R-P
Healthy	**S**ediment	**F**ixative	H-F
Unaffected	**M**ucus	**B**acteria	U-M
Diseased	**A**pplicator (Swab)		D-S

ex. Reference Site A= A.*Dstr*.1.R-P
Diseased Site B= B.A*pal*.4.D-F and B.*Apal*.2.U-M

Definitions

- "Reference" - uninfected or 'healthy-looking' colonies from areas where no corals exhibit signs of the disease
- "Healthy" - apparently healthy corals in affected sites
- "Unaffected" - areas of diseased colonies with normal appearance, distant from the lesion
- "Diseased" - margin of the lesion

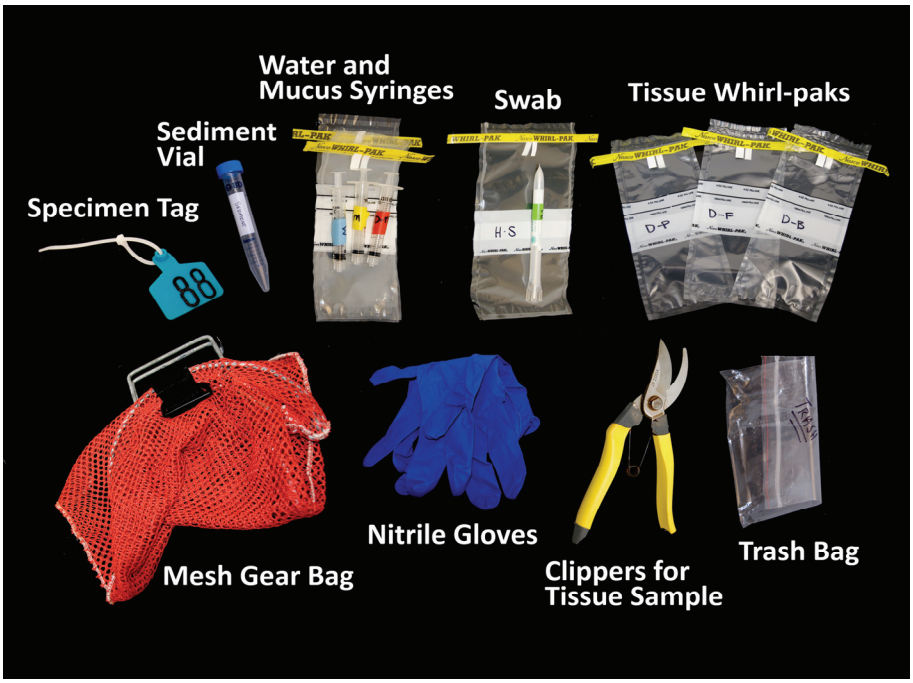


Figure 4.6.1.2 Example of components that may be used in for specimen collections during an outbreak investigation

Due to time sensitivity of some samples, such as the tissue for protein analyses, sampling should adhere to a specific order.

Within each site, samples should include:

- Water
- Sediment
- Applicator/Swab
- Syringe/Mucus
- Core or Clipped Tissue Samples for each analysis planned

4.6.2 Sediment

- Scoop sediment with sterile pre-labeled 15mL conical or similar container

This type of sample is used solely for microbiological sample analyses as a reference for microbes situated in the sediments that may be mobilized from disturbances such as storms.

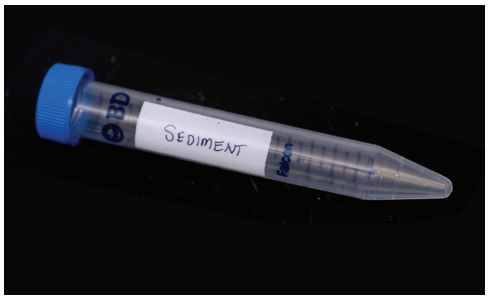


Figure 4.6.2 15cc Falcon tube for microbiology sediment collection

4.6.3 DNA Swab

- Wipe across the area to be sampled three times.

This is currently experimental and may provide less invasive sampling. The swab samples are limited to DNA analyses of surface tissue and mucus.

Figure 4.6.3 Epicenter DNA swabs

4.6.4 Water

- Collect one reference volume for each colony
- Should be equal in volume to mucus sample
- Collect in a 3cc or larger syringe

This sample is used as a reference for microbiological analyses to allow analyst to account for possible water contamination of mucus and tissue samples as well as a comparison for microbes that may be found in surrounding waters, but not primary colonizers of corals.

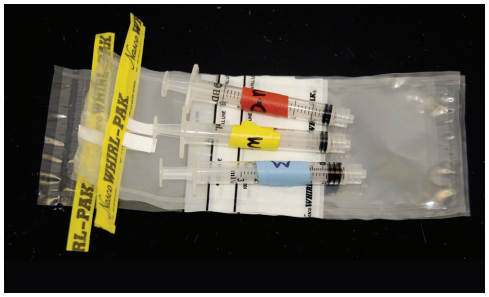


Figure 4.6.4 Syringes for water and mucus collections

4.6.5 Mucus

A sterile syringe without the needle is used to aspirate (draw in) mucus from the surface of the coral. For diseased samples, mucus is collected along the disease margins and unaffected samples across the surface of unaffected areas. If swab samples are collected, this should be done first which should provide the irritation required to obtain mucus. It is important to collect mucus already present on the colony. The diver should avoid initially irritating the colony, as mucus subsequently released by the coral will have a depauperate microbial flora community.

Mucus samples have been one of the primary types of specimens used in culture dependent and independent microbiological analyses. It seems to provide consistency across temporal and spatial sampling for microbial diversity studies. Recent work however has shown different microbial profiles are obtained depending on whether live-ground tissue or mucus is being analyzed. It appears that these two micro-environments contain different microbial communities, with tissue samples having a more diverse and robust community than mucus.

4.6.6 Tissue biopsy

Fragment/Tissue

- Coring technique- 1- 2.2cm diameter uniform disk samples of tissue + skeleton for larger colonies, using two punch sizes. **clay should be inserted after coring to minimize further damage (Roma Plastalina, no 2-from Rex Art, Miami, FL)*
- Clippers/Pliers/Garden Shears- can be used for clipping from branching specimens

Figure 4.6.6.1 Clippers used for sampling branching corals

Figure 4.6.6.2 Tools used for bolder corals. Stainless steel coring tube for histology (A) is 2.5 cm, while leather punch (B) biopsy is 1.5 cm.

Tissue samples are collected for a variety of clinical analyses. Currently available analyses include histology/histopathology, microbiology, cellular diagnostics (primarily

protein chemistry based and includes a suite of various biochemical and cell-based parameters that can be measured for building a diagnosis) and genetic or functional genomic assays.

4.7 Sample Processing for Biological Analyses

Each sample has a predetermined experimental or analytical role, which determines how each will be processed on the boat and back on land. The Sample Technician of the Support Team will do most processing.

4.7.1 SUPPORT TEAM

This team will consist of at least 2 members who will provide topside and field-lab support. The primary job of the Sample Technician is to ensure the proper handling, documentation and stabilization of each sample collected. The Logistics Chief is responsible for all dive gear and collection equipment and assists the Sample Technician.

Figure 4.7.2.1 Collection bags for healthy coral samples for H-F=fixative (histology), H-P=protein H-B=bacteria (microbiology)

Figure 4.7.2.2 Collection bags for diseased coral samples. D-P=protein, D-F=fixative (histology), D-B=bacteria (microbiology)

4.7.2 PROCESS TIME SENSITIVE SAMPLES FIRST

- **Tissue for Protein** (H-P, U-P, D-P) samples should come to the surface in dark bags or covered (e.g., glove) to protect them from light for light sensitive assays. They are time sensitive and need to be processed in a dark or shaded area. Mucus should be rinsed by swishing in seawater, dabbing on Bounty™ paper towel (or lint-free paper towel), and placing in a new, prelabeled Whirlpak™. Since Whirlpak™ bags are prone to shattering at liquid nitrogen vapor temperatures, the bags are wrapped in aluminum foil with an identifying label on the outside and placed immediately into a dry shipper. **Do not write on aluminum foil** as it is not permanent, use labeling tape or cryotags and waterproof marker. The time interval between collection and freezing should be approximately 15 minutes, longer than this will exclude certain cellular diagnostic assays due to creating artifact by changes in the sample.

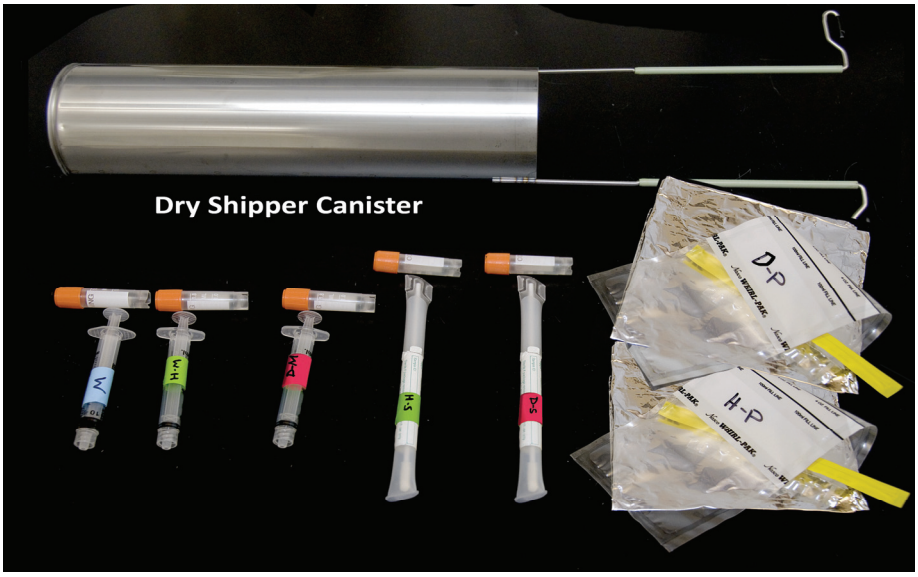


Figure 4.7.2.3 Summary of samples to be frozen showing the packaging used for freezing.

- **Tissue for Histology -** The tissue biopsies collected from **H**ealthy, **U**naffected portion of diseased colony and the **D**isease margin (**H-F, U-F, D-F**) are placed in bags or tubes underwater and on reaching the boat, if transport of fixative is logistically sound, the samples are immediately placed in a 50cc polypropylene tube containing approximately 25 mL of an appropriate fixative for a 2cm punch biopsy or an approximately 2-3 cm branch (if larger, the fixative volume should be increased in proportion). When fixative transport is precluded, histological samples should be stored in bags or a container containing seawater and securely stored to minimize stress until a destination for fixation is reached. We routinely use Z-fix (Anatech Ltd.) diluted 1:4 in sterile artificial sea water (ASW; 35ppt) and held at ~25°C, because of the ability to retrieve intact DNA from the samples for subsequent molecular and immuno-staining. The ratio of tissue to fixative should be at minimum 1:10 (1:20, preferred). **DO NOT FREEZE THESE SAMPLES.**

  Alternatively seawater-buffered formalin can be used for fixation of corals for light microscopy and formalin is generally available at marine labs, hospitals and veterinary clinics. This is prepared by filtering either natural or artificial seawater and diluting formalin stock (37.5% formaldehyde) 1 part formalin to 9 parts filtered seawater. The samples are fixed from 4 hrs to overnight then rinsed in tapwater and stored in 70% ethanol or alternatively can be stored in the 3.75% formalin-seawater.

  For shipping Kim-wipes™ or other lint-free paper is saturated with the preservative (e.g., 70% ethanol) and stuffed into the tube. This stabilizes the samples and keeps them moist, while avoiding shipping tubes filled with a hazardous material.

These samples are planned for light microscopy analyses. Electron microscopy requires different stabilization and processing and is not covered here.

- **Tissue for Microbiology** (H-B, U-B, D-B) should be kept in a Whirlpak™ with sterile 35 ppt artificial sea water added if needed, keep at ambient temperature in a cooler with local seawater. Upon return to shore, homogenize tissue and skeleton with sterile mortar and pestle (with its own mucus), flash freeze half of homogenate, and culture bacteria on marine agar or other desired media, with other half of homogenate.

Figure 4.7.2.4 Equipment used in processing tissue samples for microbiology. These include a mortar and pestle for grinding fresh tissue, device to flame sterilize spreading rods, agar plate for culturing bacteria and cryovial for freezing ground tissue sample.

- **Swabs or Applicators** (H-A, U-A, D-A), if they are Whatman FTA™ type swabs, should be wiped on the card, and then the tip should be broken off and stored in a 15 cc tube or cryovial. Other types of swabs (Epicenter, Madison WI) simply need to be broken off and the tip stored. Cards can be stored in a reclosable food storage bag (e.g., ziploc™) and shipped at ambient temperature; swab tips should go in the cryovial. ***Alternative storage to freezing is being investigated using sodium chloride saturated dimethylsulfoxide (DMSO).*

- **Mucus** samples which were collected in a syringe without needle need to be split: Half should be placed in a cryogenic vial and immediately flash frozen in a liquid nitrogen dry shipper for molecular analyses. The other half should be kept in screw top vials at ambient seawater temperature and cultured on marine agar media as soon as possible for microbiology. (See Support Team Processing Guidelines Form in Appendix V).

- **Surface sediment (**H-S, D-S) - loosen cap and attempt to remove as much water as possible. Leave about a 2 cm gap between sample and cap, cap tightly and freeze in dry shipper.

- **Water** (H-W, D-W) should be split into two samples. Half can be transferred from the syringe to a 2.0 mL cryogenic vial and placed in the dry shipper. The other half should also go in a 2.0 mL vial, but be kept at ambient temperature for culture-dependent methods.

The other roles of the Support Team at this point are to catalog these samples, track and label all samples, label and link digital photos to samples, download GPS coordinates and upload to GIS (if available), and prepare to ship time-sensitive samples.

4.8 Sample Shipment

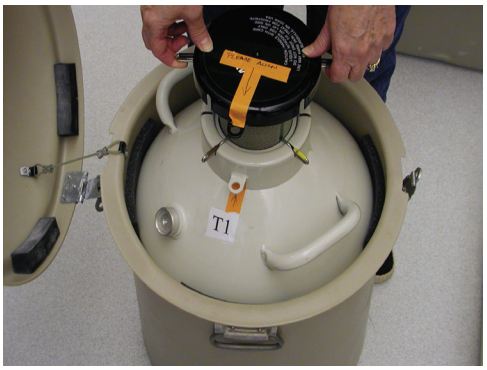


Figure 4.8 Dry shipper. Note lid and canister alignment are critical to ensuring proper seal.

The Planning Chief or Incident Commander, in collaboration with the Regional Coordinator and National Coordinator should make prior arrangements with the appropriate diagnostic laboratories to conduct the analyses, and provide advanced notification with likely dates of sample arrival before conducting the response. These dates should be confirmed before shipping samples. The Incident Commander should also follow up with the lab to ensure arrival of samples to the lab and to the appropriate person.

Samples should be placed in appropriate packaging to ensure safe delivery, avoid leaks, and fines. Each shipment should be labeled as non-regulated material to avoid concerns by the currier or inspection agents (e.g., customs). Dry ice and dry shipper labels should be used where appropriate.

4.9 Permits

It is imperative that all biological samples are collected and shipped under appropriate permits, and relevant documentation is included with samples.

4.9.1 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Permits

CITES is an international agreement between 166 governments to ensure that international trade in specimens of wild animals and plants does not threaten their survival. Under CITES, a species is listed at one of three levels of protection, each of which has specific restrictions on trade: no commercial trade is allowed in Appendix I species, while trade is allowed for Appendix II species when accompanied by an export permit that indicates the species was legally acquired and trade is non-detrimental. Coral reef species currently listed in CITES on Appendix II are 1) all corals including scleractinian (stony) corals, hydrozoan corals (*Millepora* and *Stylaster*), organ pipe coral (*Tubipora*), and blue coral (*Heliopora*), 2) all antipatharian (black) coral species, 3) giant clams (*Tridacna* and *Hippopus* spp.), 4) queen conch (*Strombus gigas*), 5) all seahorses (*Hippocampus*), and 6) humphead wrasse. For stony corals, permits are required for live corals (whole colonies and pieces with recognizable corallites), skeletons, eggs/sperm/planula, live rock, reef substrate and eroded skeletal fragments greater than 3 cm in diameter. A CITES permit is also required for coral samples less than 3 cm in diameter if the corallites are discernable and allow identification of the coral to genus (or species).

When shipping coral samples from outside of the U.S. and U.S. territories to laboratories located in the U.S., shipments must include a valid export permit issued by the exporting countries Management Authority in the country of origin (or an approved equivalent form issued by equivalent national authority in the case of countries not party to CITES). The U.S. does not require import permits. CITES permits are not required for shipments between the U.S. and our territories (Puerto Rico, U.S. Virgin Islands, Guam, Commonwealth of the Northern Mariana Islands (CNMI) and American Samoa).

When shipping coral samples from the U.S. to international destinations a valid export permit issued by the CITES Management Authority in the U.S. [Division of Management Authority (DMA), U.S. Fish and Wildlife Service] must be included with each shipment; depending on the destination country (e.g., the European Union), a CITES Import Permit is also required.

Importation of fish and wildlife, including corals, must be imported at one of the 14 Designated Ports and must be declared using USFWS Form 3-177. It is important that shipments are clearly labeled as CITES material.

When using international mail or an overnight type courier (e.g., Federal Express or UPS), shipments sometimes bypass USFWS and are delivered directly to the importer. It remains the responsibility of the importer to file the appropriate declaration form; failure to file when required is a violation of the Endangered Species Act of 1973. USFWS has simplified the declaration process by allowing the 3-177 Form to be submitted electronically (eDec) followed by mailing the original CITES permits along with a copy of the eDec confirmation page (https://edecs.fws.gov). If Certificates of Scientific Exchange are used (see below), no original CITES permits need to be sent to USFWS.

As an alternative, the CITES Secretariat has endeavored to streamline the permitting process for scientific samples by encouraging scientific institutions to register for Certificates of Scientific Exchange (COSE). The International Registry of Coral Pathology (IRCP) in Oxford, MD, administered by Dr. Shawn McLaughlin (shawn.mclaughlin@noaa.gov) and the Coral Disease and Health Consortium, administered by Dr. Cheryl M. Woodley (cheryl.woodley@noaa.gov) in Charleston, SC hold COSE permits for exchange of histological materials. To facilitate such exchanges, investigators are urged to work with their local scientific institutions to register for a COSE with the CITES Management Authority of their country (or approved for this purpose by an equivalent national authority in the case of countries not party to CITES). A one-time application and minimal fee (or waived in certain cases) permits exchange of specimens among registered institutions in lieu of filing CITES export or import permits for each shipment. COSE authorizes non-commercial loan, donation, and exchange of legally acquired scientific specimens between any institutions registered for this purpose. In the U.S., application is made by submitting USFWS Form 3-200-39 to the Division of Management Authority (http://www.fws.gov/). Upon approval, the institution is assigned a registration number and added to the list of registered institutions. Fixed and embedded specimens and micro-slides prepared from legally obtained corals may then be shipped from a registered institution to a registered institution without application for additional

CITES permits. Legally obtained Appendix II specimens may be imported into the U.S. by simply entering the registration number of the importer and exporter on the USFWS claim form. This significantly reduces the time, effort, and potential cost spent on obtaining export or import permits for individual shipments (Mc Laughlin et al. Unpublished) however, both the exporting institution and the importing institution must be registered.

4.9.2 Other Collection Permits

Each state, territory and jurisdiction has their own regulations regarding scientific collection permits. In some locations multiple jurisdictions exist, for example the Florida Keys National Marine Sanctuary's boundaries are within the State of Florida's waters and both the State and Sanctuary permits are required for the collection of coral, in others one entity issues permits. To date blanket permits have not been approved for Coral Disease Outbreak Responses, but managers acknowledge the urgency of these cases and have recommended an expedited or emergency permit process. It is important for Response Coordinators to develop a dialogue with permit offices in their region that explains the Response process and oversight provided in determining when sampling is warranted. The coordinators should also include a minimal sampling plan, rationale and projected analyses for samples that are taken during an investigation.

4.10 Types of Laboratory Analyses

4.10.1 Histology

Histological analyses are used to characterize the microscopic morphology of tissue and may help guide further investigations. They provide systematic evaluation of cellular changes that occur in tissues under normal, stressed or diseased conditions. The microscopic evaluation determines which cells or tissues are affected and whether foreign organisms (i.e., bacteria, fungi, metazoa, protistans, viruses) are present. Most

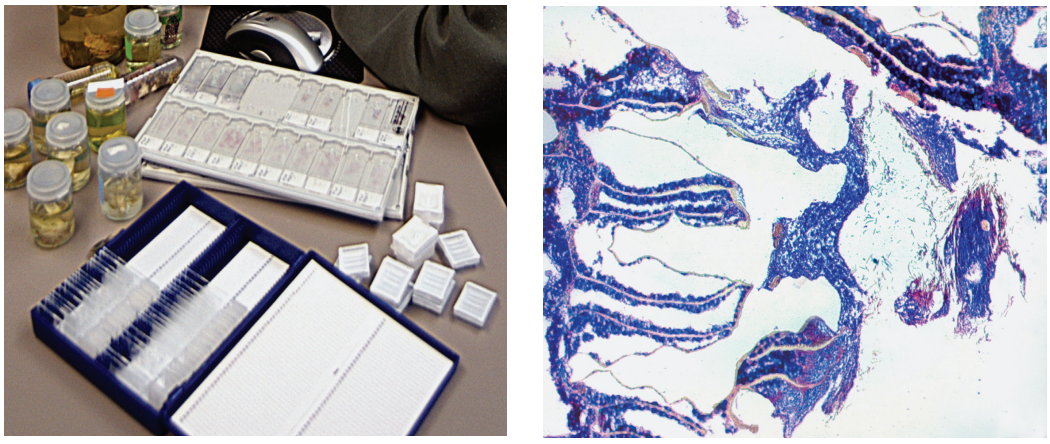


Figure 4.10.1 Histological specimens and slides (left), micrograph of coral tissue (right) *(Photos courtesy of Shawn McLaughlin)*

evaluations begin with light-level microscopy; however transmission electron microscopy can provide evidence of sub-cellular changes that are informative in understanding functional changes associated with a particular pathology.

4.10.2 Microbiology

Microbiology is one of the fundamental disciplines used in clinical diagnostics when an infectious agent has not been excluded. Coral disease research focuses heavily on microbiology ideally combining culture-dependent methods, with DNA-based technologies. While culture dependent methods are useful in identifying specific dominant cultivable microorganisms, culture-independent methods allow examination of the diversity of microbial communities associated with corals and coral mucus and shifts in these communities between hosts, species, seasons, geographic location and when exposed to different stressors. This facilitates investigations in microbial ecology, functional studies of microbial communities and differential analyses of these communities between various health conditions of corals.

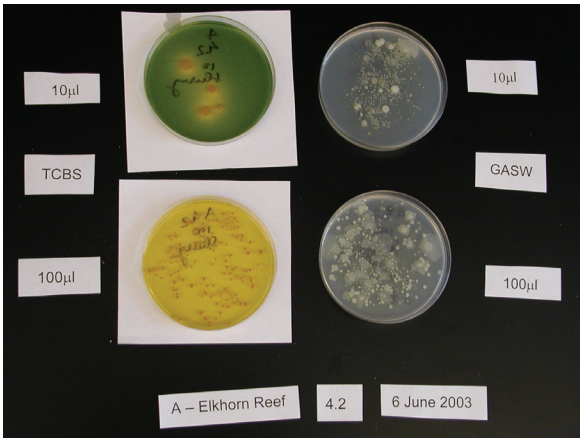


Figure 4.10.2.1 Culture dependent microbiology. Shown are samples plated on selective media (TCBS) and general media (glycerol seawater agar)

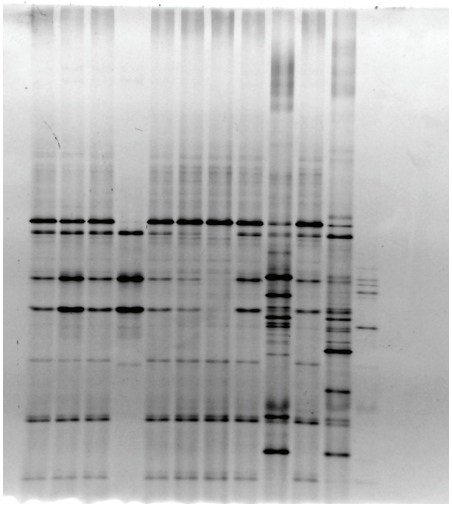


Figure 4.10.2.2 Culture independent microbiology. Example shown is microbial community profile using denaturing gradient gel electrophoresis.

4.10.3 Molecular

Molecular analyses address the formation, structure, and function of macromolecules essential to life, such as nucleic acids and proteins. Biochemical and cellular endpoints are often used in clinical diagnostic assays. Several of these have been adapted to coral and referred to as cellular diagnostics (Downs 2005a). The concept of cellular diagnostics is based on using a systematic approach to defining biomarkers of exposure, effect, and susceptibility and integrating levels of these cellular parameters into a profile that is diagnostic for certain cellular functions and disease states, and provide indicators of overall performance. The selection of cellular parameters is based on known functionality within a cell and knowledge or inference of how alterations in single or sets of cellular parameters affect cellular physiological processes. Many of these processes are involved in key metabolic pathways or cellular structural components that are

essential for cellular function and homeostasis. The behavior of these processes defines the cell's physiological condition. Thus the identification and quantification of pattern changes in the cellular endpoints provides a basis for defining health status (i.e., diagnosis) and providing a prognosis.

Molecular biology and coral functional genomics projects focus on nucleic acid, RNA and DNA. These studies are also vital to improving our understanding of the function and control of coral genes. One of the most urgent applications is to begin identifying more coral genes, understanding their expression patterns and control mechanisms. This will assist in improving our understanding of coral cellular physiology and form a stronger basis for pathology and the development of new diagnostic assays for studying and diagnosing coral disease.

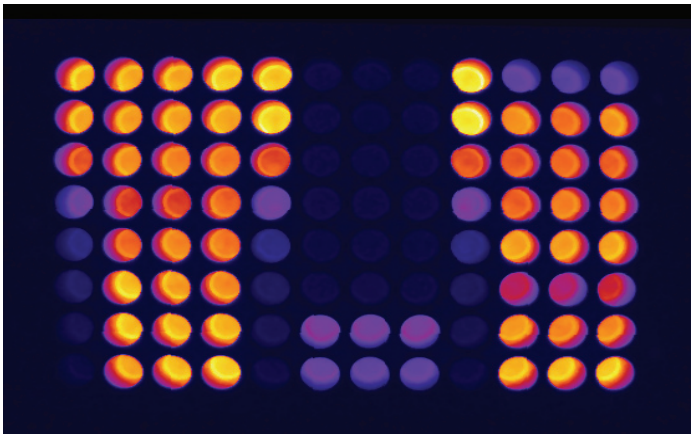


Figure 4.10.3 ELISA assay for measuring cellular diagnostic parameters. *(Photo courtesy of CA Downs)*

Chapter 5

Health and Safety

5.1 Safety Plan

The Incident Commander is responsible for completing the Site Safety Plan (SSP), which is part of the Incident Action Plan (IAP). This plan must consider personal needs, physical condition of personnel, adequacy of transportation, and potential dangers of response activities. The incorporation of diving into the investigations increases the safety concerns significantly. Boat use has its own set of regulations and requirements for safety, also. The processing of samples requires chemicals, which will need to be addressed in the SSP. The final concern is that of coral and human health when disease agents may be present.

5.2 NOAA Boat Safety Regulatory Requirements

http://www.ndc.noaa.gov/gi.html

- The Operator in Charge (OIC), Vessel Operator, or Crewmember must conduct a thorough safety briefing with all embarked personnel prior to getting underway. The briefing shall include general vessel familiarity and the locations of all safety systems and equipment carried aboard (fire extinguishers, life rafts, life rings, personal floatation devices, immersion suits, Emergency Position Indicating Radio Beacon (EPIRB), etc.). The embarked personnel shall be apprised of the procedures to follow during fire, abandon ship, man overboard, and other emergencies. The use of a formal, written checklist detailing all of the topics to be covered during each safety briefing is strongly encouraged.

- NOAA's National Ocean Service VESSEL POLICY FOR SMALL BOATS can be used for further guidance, references, directives, and details in applicable Program Operational Risk Assessments, a boat's Vessel Operations Manual (VOM), Original Equipment Manuals (OEM), or applicable United States Coast Guard (USCG) and federal regulations.

- Personal Floatation Devices (PFD's), signaling devices, and standard survival equipment and rescue devices must be available and in serviceable condition on the boat. In addition, the Safety Plan must include emergency communications procedures, man overboard rescue and in-water survival techniques, and fire fighting procedures.

- Ideally, all Operators in Charge, Vessel Operators, and Crewmembers will have current Red Cross or equivalent certification in cardiopulmonary resuscitation (CPR), including the use of Automated External Defibrillators (AED), Oxygen

Administration, and First Aid. Inspection records for the vessel should be copied and examined for any deficiencies.

- The OIC is responsible for reviewing and being familiar with both prevailing and anticipated weather conditions for the area in which the mission is planned. The OIC shall obtain a briefing by a qualified meteorological forecast service (i.e. NOAA weather radio, National Weather Service website, calling local Coast Guard for conditions, etc.). The briefing information shall consist of, at a minimum, current weather, sea state, trends, and forecasts for the departure location, proposed route, destination, and any alternate working areas.

- Based on weather and sea state forecasts, the OIC will determine if conditions are suitable for operations. The OIC has the authority to cancel operations if he/she determines that personnel safety or the safety of the vessel will be subject to unnecessary risk. A float plan must be submitted and will include contact information for all members of the response.

5.3 Dive Safety

An Emergency Dive Plan should be written to include the following:

- Name, phone number and relationship of person to be contacted for each diver
- Name, phone number, and transport plans to the nearest operational recompression chamber
- Nearest accessible hospital
- Available means of transport
- Number, depth, and duration of proposed dives
- Location of proposed dives
- Estimated depth and bottom times anticipated
- Decompression status and repetitive dive plans
- Any hazardous conditions anticipated

Divers must have appropriate dive certifications and letters of reciprocity determined by the organization in charge of the response in order to participate in a response dive. A dive master will plan and supervise dives according to the rules of the organizing institution and capabilities of the participating divers. Participating divers will adhere to all aspects of the plan, (provided it does not violate the rules of the divers' institution). The buddy system that requires at least two divers in constant communication on a simultaneous dive is a requirement of all response divers.

5.4 Precautions in the Field

Coral Health & Safety Recommendations from the CDHC

In recognition of the increased prevalence of coral disease occurring worldwide, the Coral Disease and Health Consortium (CDHC) has proposed guidelines for scientists and researchers going into the field to collect specimens where infectious agents may be present. Medical and veterinary containment measures may be easily applied to potentially infectious disease outbreaks in the aquatic environment and should be included in each response activity. The guidelines, listed below, were developed by veterinary pathologists who specialize in disease investigation and epizootics to outline preventative measures and limit the possible spread of infectious agents.

A first general response to an epizootic and epidemic is to *quarantine.* Limited access to an area can help prevent potential spread of disease agents to unaffected areas.

1. 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. Do not visit the reference site (a site with no signs of disease) after diving a diseased site without first decontaminating dive gear.

Remember: Movement should always be from "clean" to "dirty

2. Sterilize equipment/instruments between sampling (or use separate equipment/ instruments for each sample collected). Change gloves between samplings. Sanitization may be achieved by using a simple bleach solution soak (5% solution for 5-10 minutes) followed by a freshwater rinse.

3. When collecting samples, take care to prevent small pieces from falling or floating away from the sample site. Each sample should be placed into a separate clearly labeled container. Never open a sample container from one site in another area. Be sure to document the specifics of the collection site. Information should include location, morphology of the change observed (e.g. severity, area of involvement, color change, texture, patter of change, skeletal damage). Identify the specific area of tissue collected (i.e. along the margin between affected and unaffected tissue; apparently healthy tissue).

4. The responders (divers) should consider themselves and their equipment as potential vectors of disease to other locations. To minimize this risk, ALL equipment should undergo a simple sterilization process subsequent to entering an infected area or before moving to a new location. As with any disease agent, the responder should also take care to thoroughly shower with disinfectant soap prior to moving to a new location.

5. Biocontainment procedures are to be used for handling any live material taken from affected areas

5.5 Materials Hazards Information

A Material Safety Data Sheet (MSDS) shall be available for all hazardous materials carried aboard. Each vessel that is proposed to carry hazardous material must first meet all storage requirements, must have spill response kits on board, and must adhere to all NOAA hazardous material regulations prior to leaving the pier.

Chemicals used during an investigation will be documented and accompanied by appropriate MSDS forms. The crew will be properly trained in handling practices for hazardous materials (i.e. z-fix, gluteraldehyde, liquid nitrogen, dry ice). The OIC should be informed of potential hazards in order to plan effectively for any emergencies.

Glossary of Terms

Bleaching: Loss or degradation of zooxanthellae due to biotic (bacteria) or abiotic (e.g., temperature, UV radiation, salinity, toxicants) causes.

Coral Disease Outbreak Investigation: An unusual disease occurrence has been reported. Regional Coordinator is notified and implements a level of investigation he sees fit. Sampling procedures in an investigation are standardized, and effort is made to determine the severity and causality of the outbreak.

Disease: Any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects, or combinations of these factors. (Wobeser 1981).

Enzootic: Disease that occurs in a population at a regular, predictable or expected rate, usually low frequency, affecting only a few animals at any one time; similar to endemic, used for human populations

Epizootic: Disease that appears at a time or place where it does not normally occur or with an abnormally greater frequency for a specified time; similar to epidemic, used for human populations

Expert Working Group: Complied by the National Coordinator, this group of coral disease experts assists the National Coordinator in decisions during an outbreak investigation, i.e., when to close the case/ implement a response plan.

Growth Anomalies: Abnormal growth and development, including hypertrophy, hyperplasia, neoplasia, tumors

Health: A continuum between "… absolute health (a state in which all functions are optimal) and death, which occurs when functions are so severely compromised that life is impossible. Between the two points there is a region of relative health that blends imperceptibly into a region that we can define as disease." (Wobeser 2006).

Holobiont: (coral) A collective term referring to the totality of a coral animal, its endosymbiotic zooxanthellae, and the associated community of microorganisms.

Hyperplasia: an increase in the number of normal cells in normal arrangement in a tissue or organism, increasing its size.

Hypertrophy: Occurs in tissues or organs due to an increase in the *size* of cells, while the number stays the same.

Incidence: Frequency with which the disease has increased from base line (new occurrence)

Incident Command System: Management tool used by Federal (and State) emergency responders in response to a planned event, natural disaster, or terrorist attack. It can be used to investigate the causes/ prevention measures for an emergency, and can be applied to an Outbreak Investigation (http://www.nrt.org/(2000a)).

Incident Commander: In a Level III response, oversees response teams in order to take samples for detailed analyses, makes arrangements for logistics, lodging, boats, support staff, shipping of samples, laboratory use, permits, etc. and is designated by the Regional Coordinator.

Infectious diseases: Partial and whole colony mortality caused by bacteria, fungi, viruses and other microorganisms.

Level I Response: An unusual observation is made in the field. Regional Coordinator interviews the observer.

Level I case closed: Regional Coordinator determines the case is closed after the interview for reasons such as, but not limited to, the case already being reported, lack of credibility given to the initial observer, determination that it was a non-disease observation, or if the Regional Coordinator is never able to contact observer.

Level II Consultation: Used to determine the need for a Level II data collection trip and requires the following be considered: strength of observation; magnitude supported by surveys, photos, and prevalence data; and availability of boats and staff in the area with specific knowledge.

Level II Recommendation: May be made if the report constitutes a new observation, if more information is needed, if the species affected is one at particular risk, if the magnitude appears to be large, if there is a change from earlier reports, or if there are pictures needed to validate the report.

Level II Response: Reconnaissance team of knowledgeable divers observes the incident to investigate its severity and report findings to the Regional Coordinator.

Level II Case Closed: After a Level II response, the Regional Coordinator determines that the case is closed for the following reasons: observations were not supported during Level II response; the disease is within normal (known) background levels; non-diseased agent (i.e., boat trauma, hurricane damage) caused the issue; the decision to refer the case to another response team (bleaching, grounding, fish kills); or adequate information was obtained in Level II.

Level III Consultation: Aids the National Coordinator and expert working group in their decision to launch a level III response, and includes considerations such as: the strength of the Level II observations; the magnitude (distribution (multiple reefs), frequency, multiple species, higher than expected proportion of colonies affected or

mortality rates); the apparent occurrence of a new/unusual condition; temporal irregularity; the relative importance of species at risk; or potential population and/or community impacts.

Level III Response: Full scale investigation to determine causality and clarify severity; launched upon the recommendation from the Regional Coordinator and confirmed by the National Coordinator.

Monitoring of Coral Disease: A routine, local effort to sample or observe coral. Disease may or may not be present, but it is not considered a direct threat. Sampling techniques may vary. ***A severe disease outbreak has not been reported***

National Coordinator: Versed in coral disease; Serves as central contact for all Regional Coordinators, collates all reports of verified Level I and II investigations submitted by Regional Coordinators, and convenes the Expert Working Group to make decisions for a Level III response

Non-infectious diseases: Physiological and morphological (e.g., tissue loss or discoloration) changes due to agents such as toxins or toxicants, sedimentation, pollution, and other environmental stressors

Parasitic infections: Infestation by protozoans (e.g., ciliates, amoeba), metazoans (e.g., trematodes, flatworms, flukes) or parazoans (e.g., sponges)

Prevalence: Current number of the population affected by the disease (old and new)

Proficiency Drills: In class and field exercises to review each response step and consist of varying scenarios aimed to determining a team member's ability to conduct their assigned tasks

Regional Coordinator: Knowledgeable about coral disease and its occurrence in his/her region; Coordinates communication between scientists, managers and the public in order to assess the threat of an incident, implement control and prevention procedures, determines the amount and type of data needed to recommend further action. Regional Coordinators conduct interviews with initial observers after a Level I observation, and evaluate the need for a Level II response. Following this, Regional Coordinators recommend to National Coordinators a need for a Level III investigation, or to close the case. Current regions include the Southeast United States, Gulf of Mexico and Florida coast; the Caribbean; and the Pacific.

Surveillance: Systematic collection, analysis, and interpretation of health data

Trauma: Physical damage (e.g., groundings, fish bites)

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Appendices

Appendix I. Regional Coordinator Interview Checklist

Interviewer Contact Information
Name _____
Address _____
_____
_____
Tele _____
Email _____
Affiliation _____
Date of Interview (mm/dd/yy)_____

Was Case Number Assigned? Yes_____ No_____; Case Number _____

Contacted 'Observer'? Yes_____ No _____ date (mm/dd/yy)_____
Comments_____

Verified Level I Report? Yes_____ No _____
Comments_____

Asked if there were unusual observations? Yes_____ No _____
Comments _____
_____

Verified where Observer obtained Level I form? Yes_____ No _____
Comments _____

Was Level II information acquired? Yes ____: Complete ___, Partial ____; NO ___
Comments_____
Level II Form attached? Yes_____ No _____

Was Level II Case Worker Assigned?
Name _____
E-mail _____

Contacted Level II Observer? Yes_____ No _____
Comments _____ date (mm/dd/yy)_____

Contacted Advisory Team? Yes_____ No _____
Comments _____ date (mm/dd/yy)_____

Type(s) of Feed back: telephone call ____; E-mail _____; letter_____; webpage info_____
Comments _____
_____

Case Identification Number Generation SSCC## - mmddyy – XXX,#### (Group ID) - (Date) - (species, sample #)	
Group ID SS – two letter State Designation (e.g. FL, PR, VI, HI) CC - two letter City Designation (to be generated)	**Species, Sample Number** XXX - use first letter of the genus and first two of the species, e.g., Porites lobata, Plo ### – #### designates event #

Appendix II. Level I Data – Coral Disease Event Report

Case ID # _____ (Administrative Use Only)

*Required Information

***Observer Information** Name _____ Address _____ _____ _____ Tele _____ Email _____ Affiliation _____ Date of Observation (mm/dd/yy) _____	**Location of Suspected Disease Event** *Geographic locality (site, city, county, state) _____ Name of Reef _____ Reef Type if known _____ GPS coordinates _____ _____ How many times have you dived on this reef? _____
Occurrence Details Single location Yes___ No___ Unk___ Throughout Reef Yes___ No___ Unk___ Multiple Reefs Yes___ No___ Unk___ How many coral colonies? _____ Coral types affected (circle) 1 2 >3 *Recent Change Yes___ No___ Unk___ *Previously observed? Yes___ No___ Unk___	**Species Affected** Genus _____ Species _____ Common name _____ *Describe types _____ (e.g., branching, boulder)
***Description of Affected Coral** Color change Yes___ No___ Unk___ Tissue loss Yes___ No___ Unk___ Skeletal damage Yes___ No___ Unk___ Growth anomaly Yes___ No___ Unk___ Describe _____ (any specific information you may have)	**Data Collected** Sea state _____ Water temperature _____ Water clarity _____ Photographs _____ Depth _____

***General Description** of what you saw:

_____

_____

_____

Thank you for filling in this form, your information will be used to assist in the study of coral disease. For additional information please see the webpage: http//:www.coral.noaa.gov/coral_disease/cdhc.shtml It would be helpful to know where you obtained this form:

_____

(e.g., Web, Dive Shop, Nat. Park Serv., Nat. Mar. Sanc., Other-describe)

Appendix III. Level II Data – Coral Disease Event Report

Field Identification Number _____ (see instructions below)

Level II Observer (or…Interviewer) Information: Name _____ Address _____ _____ _____ Tele _____ Email _____ Affiliation _____ Date of Observation (mm/dd/yy) _____	**Reef Descriptors:** Reef Name _____ Reef Type _____ Depth _____ GPS coordinates _____ Semi-quantitative extent of the affected areas _____ Adjacent Reef Information _____ _____ Location relative to affected reef: _____
Background Information: Case ID # _____ (provided by Regional Coordinator) Level I Data Sheet attached Yes ____ No ____ (provided by Regional Coordinator) Date of Level I Observation (mm/dd/yy) _____	**Data Collected on Boat:** GPS coordinates _____ Weather _____ Water temperature _____ Sea state _____
Characteristics of Affected Reefs: 1. Are corallivores present (give species)? _____; if absent go to 2. 2. Is there overgrowth (competition)? yes _____; if no go to 3. 3. Suspect disease present? no ___; if yes, answer following questions: a. Reef type (circle appropriate descriptors): inshore, offshore, bank, patch, barrier, emergent, submergent b. Provide any missing information in Level I Report _____ c. Dominant or other coral species affected? Yes (identify) _____; No___ d. Other marine species affected? Yes (identify) _____; No___ e. Are key indicator species present? Yes (identify) _____; No___	f. Reef relief – high _____; low _____ g. Rugosity – high _____; low _____ h. Cover – percentage _____. i. Species associations _____. j. Macroalgae? present _____; absent _____. k. Distribution of affected corals _____ l. Prevalence of affected corals _____ m. Recently dead colonies – if yes _____ is there algal cover? If yes ____then what is the estimate of days of algae growth (7-10 days?); No _____.

Level II Data: Coral Disease Event Report - Continued

Coral Species Affected:	**Data Collected at Reef Site:**
Genus _____ Species _____ Common name _____ Describe types (e.g., branching, boulder) _____ _____ **Coral Species Not Affected:** **Describe** _____ _____ _____	Water clarity _____ Map drawing of site _____ Quantitative transects/survey of reef area _____ Signs of recent reef disturbance _____ Photographs _____ Videos _____ Samples (circle appropriate selections) a. diseased coral tissue b. healthy coral tissue near to diseased tissue c. healthy coral tissue from another coral d. other species e. water f. nearby sediment/soil/sand Depth _____
Description of Affected Coral **Tier 1:** Color change Yes___ No___ Unk___ Tissue loss Yes___ No___ Unk___ Skeletal damage Yes___ No___ Unk___ Growth anomaly Yes___ No___ Unk___ Describe _____ (any specific information you may have)	**Description of Diseased Tissue on Affected Coral** **Tier 2:** Shape _____ Color _____ Size _____ Distribution _____ Number _____ Polyps _____
Additional Information - Provide Narrative Description of Diseased Coral _____ _____ _____ Indicate whether you used Http://usgs.madison.gov/coral_disease_characterization to develop a preliminary diagnosis of the disease: Yes_____ No _____	

Thank you for participating in the Level II response to a recent report of coral disease and collecting information/ samples in support of the CDHC Rapid Response to Coral Disease. Your information will be used to further the study of coral disease. For additional information please see the webpage: http://www.coral.noaa.gov/coral_disease/cdhc.shtml

Field Identification Number Generation

SSCC## - mmddyy –XXX,###XX
(Group ID) - (Date) - (species, sample #)

Group ID	**Species, Sample Number**
SS – two letter State Designation (e.g. FL, PR, VI, HI) CC - two letter City Designation (to be generated)	**X**XX - use first letter of the genus and first two letters of species, e.g., Porites lobata, Plo ###XX – ### designates event # and XX is sample designation by letters, e.g., AA, AB

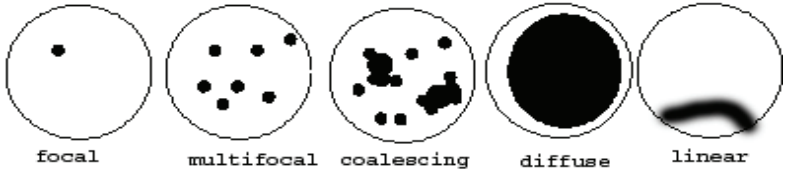
Appendix IV. LEVEL III - Coral Disease Assessment Form

Date: _____ Site: _____ Recorder: _____ Transect: _____ Depth:____ Heading:_____
Comments:_____

Spp.	Diam. (cm)	Lesion Type	Distribution F, M,C, D, L	Location B, M, A	Lesion Size	Lesion color	Lesion margin	Lesion Severity	Duration A, S, C	Comments

Lesion Location

Lesion Distribution



Lesion Margin: Smooth (S), Irregular (I)
Lesion Severity: Mild (<10%), Moderate (10-24%) Severe (25-49%), Extreme (50-100%)
Lesion Duration: Acute (no algal colonization Subacute (filamentous algae) Chronic (gradation of algal types)

Appendix V. Support Team Processing Guidelines Form

	Analysis	Processing	Preparation to Ship
H-P Tissue **U-P Tissue** **D-P Tissue**	Molecular (protein/DNA)	Time sensitive- IN SHADED OR DARK AREA: Rinse mucus by swishing in seawater, dab on Bounty, place in new pre-labeled Whirlpak™	Wrap in aluminum foil, label with waterproof marker or pre-printed cryotags, place in dry shipper
H- F Tissue **U-F Tissue** **D-F Tissue**	Histology (fixative)	Immediately preserve by transfer to a 50 cc polypropylene tube with approximately 25 mL of an appropriate fixative, such as Z-fix (Anatech Ltd.) diluted 4:1 with sterile ASW (35ppt) * 1:10 tissue: fixative ratio	Hold at ~25°C (Store in cooler) DO NOT FREEZE
H-B Tissue **U-B Tissue** **D-B Tissue**	Microbiology	Keep in Whirlpak™ add sterile artificial sea water if needed, keep at ambient temperature in cooler filled with local seawater	Upon return to shore, homogenize tissue and skeleton with sterile mortar and pestle – Flash freeze half of homogenate – Culture bacteria with other half of homogenate
H-S Swab **U-S Swab** **D-S Swab**	Molecular and Microbiology	Epicenter type swab- break off tip and put in cryovial, FTA type swab wipe on card, then break tip and store in 15 cc tube or cryovial	Put cryovials in dry shipper, store card at ambient temp. in Ziploc or other container
H-M Mucus **U-M Mucus** **D-M Mucus**	½ Molecular	Should be placed in a container, such as a cryogenic vial	Immediately flash freeze in a liquid nitrogen dry shipper
	½ Microbiology	Should be kept at ambient seawater temperature, possibly in screw top vials	Culture on media as soon as possible (2-3 hours)
H-Sediment **D- Sediment**	Microbiology & Molecular	Top-side- Invert tube- shake- loosen cap and decant water into 2 mL cryovial Leave gap ~2 cm between sediment sample and cap	Cap sediment tightly and freeze in dry shipper Store liquid at ambient temperature and plate in lab
H-Water **D- Water**	½ Molecular	Transfer from syringe to 2.0 mL cryogenic vial	Place in dry shipper
	½ Microbiology	Transfer from syringe to 2.0 mL cryogenic vial	Keep at ambient temperature for culture dependent methods

Appendix VI. Pathology Sample Submission Form

International Registry of Coral Pathology

NOAA, NOS, NCCOS, CCEHBR, Cooperative Oxford Laboratory
904 South Morris Street Oxford, Maryland 21654 USA
(410) 226-5193 (Phone) (410) 226-5925 (FAX)
email: shawn.mclaughlin@noaa.gov or kathy.price@noaa.gov

Accessions are solicited in the following order of preference: 1) slides, photos and publications 2) tissue blocks 3) fixed tissues

Instructions for submission of specimens:
1) Each specimen must be accompanied by a submission form. If you are submitting multiple specimens, please use separate forms, which may be obtained by contacting the above addresses or downloading from the website.
2) Please complete as much information as possible. Items in **BOLD** are required for the specimen to be accessioned into the IRCP. (enter "0" or "none" if applicable). Use additional sheets as needed.

NAME: Phone:
Affiliation: Alternate Phone:
ADDRESS: Fax:
email:
CITY: **STATE/PROVINCE:**
ZIP/POSTAL CODE: **COUNTRY:**

DATE submitted/shipped to IRCP: **DATE IMPORTED** to USA (if applicable):

MATERIALS SUBMITTED:
Gross specimen description (include description of lesion(s), if any, such as size, color, texture, morphology, location/pattern/timing of tissue loss or disfigurement, tissue margin appearance, etc.):

Number of epoxy / other blocks: B&W photographs: TEM photomicrographs: Other:
Number of paraffin blocks: 35 mm slides: SEM photomicrographs:
Number of microscope slides: Color Prints: Digital Images:

Publication (s) / reprint(s) associated with this specimen? (Reprints attached? Yes ☐ No ☐)

Specimen Information:
Specimen ID number (if any)
Common name(s)
Genus species
Date collected Approx. time collected
COLLECTION PERMIT NUMBER*
Date permit issued
Permit issuing authority
Name of person who was issued permit:

Collection information:
Body of water**
Continent / Island
Country
State / Province
City
Locality
Latitude
Longitude

Please attach copy of Fish and Wildlife declaration form if imported.

*** If the specimen originates from outside the USA or its territories and is CITES listed** (i.e. scleractinian coral), shipments must be labeled "**CITES MATERIAL**" for customs and USFW inspection. Contact coral.registry@noaa.gov prior to shipping materials.

** If a captive specimen, please attach additional information on type of tank (size, filtration, lighting); other tank inhabitants, length of time in captivity, etc. If you have information on the coral's place of origin, please include.

Contributor diagnosis (if any) / possible etiology:

Approximate colony size: Collection method:

Water depth at collection site:

Please attach any water quality data, if available (pH, salinity, temperature, dissolved oxygen, chlorophyll extraction, etc.)

Fixative: Fixation method (i.e. time between collection and fixation; time in fixative; rinses; etc.):

Shipping / storage solution (if different from fixative) [1]:

Decalcification solution:

Decal procedure (i.e. 8 changes neutral EDTA over 4 days; 24 hrs. water wash):

Additional observations [2]: (Attach case reports or other written summaries, if available.)

General condition of colony (healthy; bleached; swollen; sloughing mucus):

Overall colony coloration (healthy or "normal"; bleached):

Estimated % live reef cover and coral species richness (collector's observations/comments):

Predators, parasites, or pathogens in evidence (snails; damselfish; starfish or urchins; algae):

Anthropogenic impacts (damage from divers; boat anchors; grounded vessels; dredging and/or trawling operations; sedimentation; dynamiting; other):

Habitat (back / fore reef; shallow water / bright light; deep / shaded area; cave ceiling / overhang; exposed area with surge; etc.):

Is there a background photo of surrounding reef area available?

Occurrence of recent storm or severe weather event or natural phenomenon?

Comments

Microscope slides and images are kept on permanent file. Blocks are maintained permanently unless their return is requested at the time of submission. Contributor will be acknowledged in accession catalog, website, atlas and other related publications, with anonymity respected upon request. Contact information will not be distributed without prior approval.

[1] For information regarding the appropriate packaging method for shipping, please contact the IRCP.

[2] Standardized methods for characterizing the conditions of coral reefs may be found at: www.cofc.edu/~coral/Vitareef/vitareef.htm

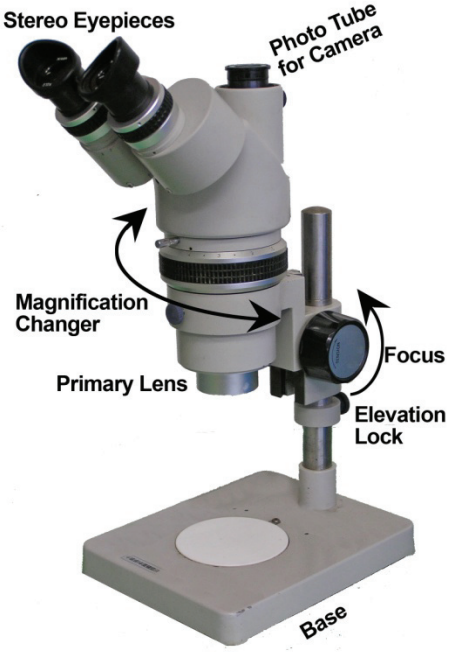
Appendix VII. List of Sampling Equipment and Supplies

Documents	**Histology Sample Processing**
Collection Permits	Seawater buffered formalin (Z-Fix, 1X aliquoted)
Travel Documents	50 cc Falcon tubes & 4ml cryotubes
	4% glutaraldehyde-2% formalin in seawater (TEM)
Surveillance & Ecological Data Collection	Sterile seawater (for fixative dilution)
Underwater digital camera still and video	Cooler & ice packs for glutaraldehyde fix
Datasheets, pencil and clip board	
Floating chains weighted	**Contaminant Chemistry Sampling**
GPS	Nitrile gloves, assorted sizes
Scale bar and identifier board for photos	Sediment jars (250-500cc) pre-cleaned EPA standard
Scooter and batteries	Teflon bags w/ sealing clips
Slates	Aluminum foil acetone-pre-cleaned
Tape measures	Stainless steel coring tube, acetone cleaned
Tags e.g. cattle tags	
Biological Sampling	**On Board General Supplies**
Dive slates	Utility knife
Data sheets and pencil	Scissors
Camera	Permanent marking pens & pencils
Mesh dive bags	Rubber bands
Tissue Collection	String
Film canisters or 50cc tubes for tissue	Clipboards
Nitrile gloves (S-XL)	Batteries for cameras and GPS
Stainless steel coring tubes (for boulder corals)	Whirl-Pak™ or Ziplock™ bags qt and gallon sizes
Clay- Roma Plastalina #2 Rex Art Miami, FL	Plastic cable ties, assorted sizes
Clippers, curved blade (for branching corals)	Clear tape
Hammer	Labeling tape
Chisel (small)	Waterproof paper
Mucus Collection	Fishing weights
Syringes with caps – 3cc, 5cc or 10cc	Kimwipes™ large and small
15cc Falcon tubes for sediment as background	Bounty™ paper towels (or other lint free brand)
15cc Falcon tubes for water column	Plastic garbage bags
DNA swabs - Epicenter	Bleach
On board Sample Preservation & Processing	**Equipment for Refurbishing Collection Tools**
Cryoshippers	Angle grinder – 4.5 inch e.g. Dewalt 402K
Marine coolers & ice packs	Grinding wheels – assorted
2ml and 4 ml cryovials	Cutoff wheels (min. 6) and adapter for attaching
Aluminum foil (tissue packaging)	Safety goggles
Labels – waterproof and cryogenic	Leather gloves for sharpening cores
	Angle file
Sample Processing Equipment	Round file
Portable vacuum pump	
Tubing, stoppers, side-arm flask (trap)	
Disposable sterile vacuum filter units w/bottle	

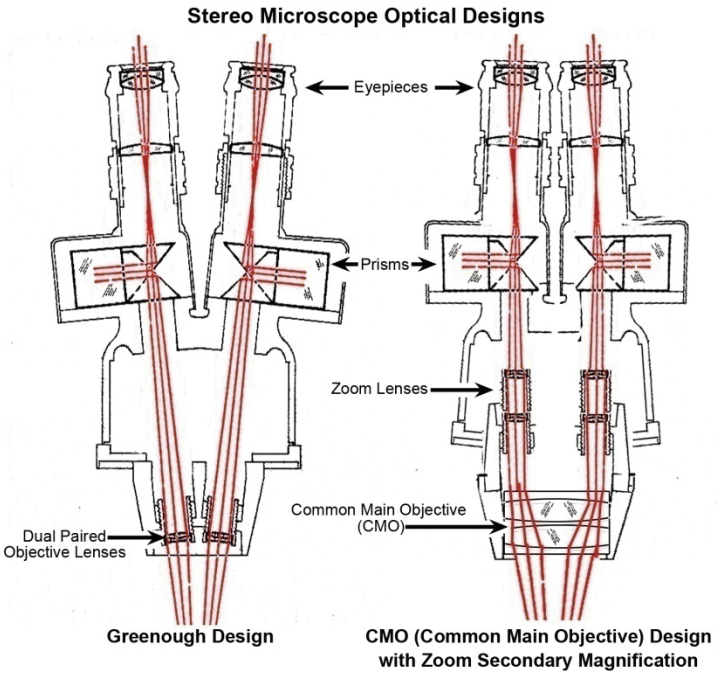
Appendix VIII. Stereo Microscopy

Introduction

The stereo microscope, also known as a dissecting microscope or operating scope is an entirely separate design from the more familiar compound microscope, and serves a different purpose. It produces a three-dimensional visualization of the sample being examined through the use of two separate optical paths so that the left and right eyes receive slightly different viewing angles which the brain interprets as a three dimensional image. The stereo microscope should not be confused with the compound microscope which is also equipped with binocular eyepieces. Both eyes see the same image in the compound microscope so the visual image is no different from that obtained with a single monocular eyepiece. In this type of microscope the binocular eyepieces provide greater viewing comfort rather than three dimensional imaging.



The design of the stereo microscope provides great working distance and depth of field which are essential qualities for this type of microscope. Working distance and depth of field are inversely correlated with resolution. The greater the depth of field and working distance the lower the resolution (the distance at which two adjacent points can be visually resolved as separate). The stereo microscope's useful magnification is up to 100X which is approximately the same magnification of the 10X scanning objective in a compound microscope. In practice, the majority of stereo microscope observations are made between 4X and 40X.



Optical Design and Magnification
Total magnification in the stereo microscope is achieved by a primary magnification which is further increased by a secondary magnification. The primary magnification of the microscope is determined by a primary objective lens which can be either a single lens

called a Common Main Objective (CMO) or a paired set of objective lenses often called a Greenough design. The main advantage of the CMO design is the infinity focus which produces two collimated light paths with parallel axes between the objective and the eyepieces that allow the easy insertion of various optical and accessory components such as camera ports and drawing tubes. This gives the greatest versatility and makes it the preferred choice for research applications. Some models have interchangeable primary lenses giving a choice of several different ranges of magnifications. The Greenough design is often smaller, less expensive, rugged, and simple to use and maintain. They are a popular choice for simpler workhorse applications which don't require a large variety of accessories.

The secondary magnification is achieved in one of three ways depending on the optical components. The simplest is fixed magnification which has a single set secondary magnification. This means that the microscope is limited to one final magnification. This is most often seen in small and highly portable scopes intended for field use; some of which can fit in a pocket. The next type is a system of selectable set magnifications controlled by a rotating drum or ring. The most complex system is zoom magnification, capable of a continuously variable magnification across a set range. Zoom systems can also have interchangeable primary lenses or use auxiliary objectives that increase total magnification by a set factor. Also, total magnification in both fixed and zoom systems can be varied by changing eyepieces.

Illumination

The most common form of illumination for a stereo microscope is reflected illumination rather than transmitted illumination. That means the light is reflected from the surface of an object rather than transmitted through an object like a compound microscope. This allows the examination of specimens that are opaque or too thick to allow light to pass through them. Stereo microscopes can use transmitted light illumination as well if equipped with a light source beneath a transparent stage or inset in the base; however the transmitted illumination is not focused through a condenser like a compound microscope. Optional illuminators are also available for reflected or transmitted dark field microscopy.

Primary Applications of the Stereo Microscope

The stereo microscope is most often used to study solid specimens or to carry out tasks such as dissection, or sorting that require a close-up view. The long working distance and the fact that specimens can be examined without any preparation or processing makes this a uniquely versatile instrument for field use. Aquatic organisms can be easily examined live in a suitable container of water.

Different models are available ranging from large sophisticated research models often equipped with cameras to small pocket size models for on-site field examination. A recent innovation has been a new class of research stereo microscopes designed especially for fluorescent imaging requiring special light sources and exceptionally high

light gathering ability. A digital camera is used which combines high image quality with the ability to image weak light signals. These microscopes are very large and extremely expensive but they have opened a whole new way of studying the often weak fluorescent material with a much larger field size and on opaque materials that would be impossible on a compound fluorescent microscope.

Choosing a Stereo Microscope

The choice of the best model for a particular application is determined by the following criteria:

The physical environment in which it will be used. Larger research grade microscopes are best used in a laboratory or on larger research vessels with electrical power for cameras and light sources, and climate control to protect more delicate optics and electronics. Smaller simpler and more robust models are suitable for use where environmental factors are less ideal. Pocket sized and preferably water proof models are best for small boats and on-foot research.

Whether imaging is required. Stereo microscopes for imaging require higher quality optics and a separate trinocular port for the camera. Ultra high resolution and low light level cameras are best used in a laboratory. The new relatively inexpensive CMOS cameras with a USB2 interface are ideal for portable use because they can be plugged into any notebook computer with basic software installed.

The primary intended use. Microscopes intended to cover a wide and varying combination of research applications should have the greatest range of magnifications and light sources. Here the CMO design should be considered first. Microscopes intended for surveying or sorting specimens can have a more modest range or in many cases a fixed magnification. The simpler Greennough design might be a better choice for this work.

The type of stand required. The standard short desk stand can accommodate specimen and container sizes over typically a 6” to 12” range. Optional taller stands are often available where a greater range is required. Most versatile is a stand and arm combination which allows a large range of vertical and horizontal movement which can be used for applications such as close-up examination of marine animals too large to place and move under a standard stand.

Budget and appropriate quality for the intended use. Stereo microscopes can cost anywhere from up to $20,000 for the most sophisticated research models to a few hundred dollars for the most basic models. Besides budget restrictions, it is inappropriate to get a very expensive microscope for a destructive environment such as exposure to salt spray since any microscope will have a short service life. The very least expensive microscope adequate to perform the required work is a better choice with frequent replacement as required.

With reasonable care stereo microscopes have long service lives and highly serviceable older models can be found at bargain prices by a careful shopper. A search on the internet will located microscope dealers that carry used equipment inventories. The AO Cycloptic, the first modern commercial stereo scope, is a robust instrument, ideal for a field station or research vessel use and can be found at very modest prices. A quick survey on EBay produced 536 stereo microscopes for sale. The majorities were new student grade scopes of questionable quality but there were also older models, some of which were top of the line in their day and now offer sophisticated performance at bargain basement prices.

Care and Maintenance of a Stereo Microscope

A well designed stereo microscope requires surprisingly little maintenance. Most problems can be prevented by some simple, common sense, proactive preventative steps. Bear in mind that cleaning optics is inherently destructive over a long period of time so preventing optical contamination is better than cleaning it off. One of the most useful microscope accessories that is too often unused is the simple dust cover. A microscope should always be covered when not in use. Special consideration should be given to the type of cover where ever there is the possibility of water, chemical or blowing sand affecting the scope.

Common dust is usually not of concern and if excessive enough to be bothersome is easily removed with a source of air, either commercial canned air, or an ear syringe. The most common type of contamination that requires prompt and thorough cleaning is finger prints. The oils in a finger print can actually etch the optical coatings on the lens. Eye makeup such as mascara can be a chronic problem in the contamination of the eyepieces. The best solution is to discourage the use of eye makeup by personnel using microscopes. Salt spray needs to be removed by the careful use of fresh water cleaning using damp clothes, never liquids that could get into the scope.

Proper cleaning of optics

1. Have proper materials on hand including good quality lens paper, a source of air and lens cleaner.
2. Always first use air to blow off the optical surface to remove any grit that could scratch the optics during cleaning.
3. Never touch an optical surface with any dry material. Always moisten the cleaning cloth or tissue with lens cleaner or use your breath to fog the lens.
4. Suitable cleaning materials include lens tissue, microcloth, or a well laundered clean handkerchief.
5. Clean in a circular motion without applying excessive force. Make several passes using a clean surface each time.
6. The use of solvents should be carefully restricted to lens contamination such as oil or mounting media that actually requires it. Never apply any solvent directly to a lens but always apply it to lens paper of a cotton swab. Shake off excess liquid before applying to the lens. Materials like oil will require the use of multiple swabs or papers as they must be discarded after each pass. Check all safety instructions for any solvent and make sure you have adequate ventilation, and personal protection as required.

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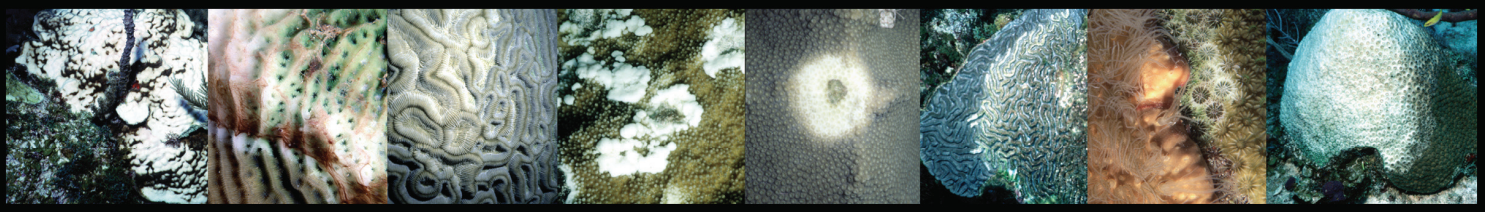

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