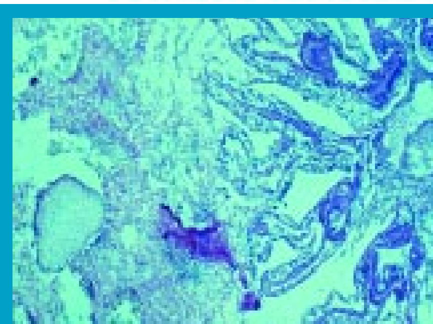
CORAL DISEASE AND HEALTH: A NATIONAL RESEARCH PLAN

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

September, 2003

About This Document

Editors' Acknowledgements - This document was prepared and printed with support from NOAA through the Coral Conservation Program and the Living Oceans Foundation. Layout and design was provided by Andrew Bruckner, NOAA Fisheries. Sylvia Galloway and Cheryl Woodley, NOAA CCEHBR and Andrew Bruckner, NOAA Fisheries compiled working group reports and provided technical edits.

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Cover Photos: *Montastraea faveolata* with white plague, Bonaire, 2001 (photo by Andrew Bruckner); diver surveying *M. annularis* for white plague in Virgin Islands National Park (photo by Jeff Miller); coral tissue destruction by algal turf on the skeleton of a colony of *Stephanocoenia intersepta*. Stained with Harris's hematoxylin and eosin (photomicrograph by Esther Peters).

Back page: Red-band disease on a sea fan; white-band disease on *Acropora palmata*; partially bleached *Diploria labyrinthiformis*; dark-spots disease on *Siderastrea siderea*; yellow-blotch disease on *Montastraea faveolata*; spot biting by *Sparisoma viride* on *M. annularis*; white plague on *Diploria strigosa* and black-band disease on *M. faveolata* (all photos by Andrew Bruckner).

Citation- Please cite this report as follows:

Woodley, C.M., A.W. Bruckner, S.B. Galloway, S.M. McLaughlin, C.A. Downs, J.E. Fauth, E.B. Shotts and K.L. Lidie. 2003. Coral Disease and Health: A National Research Plan. National Oceanic and Atmospheric Administration, Silver Spring, MD. 72 pp.

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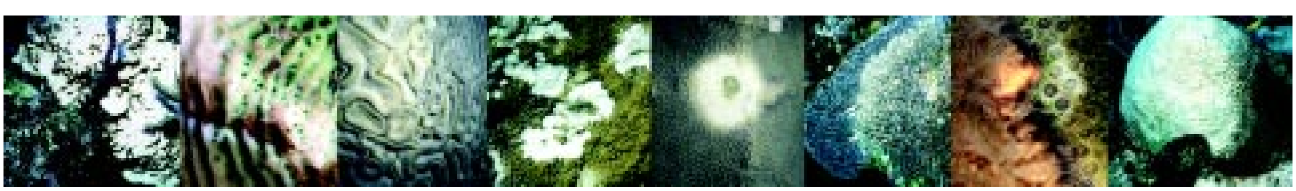
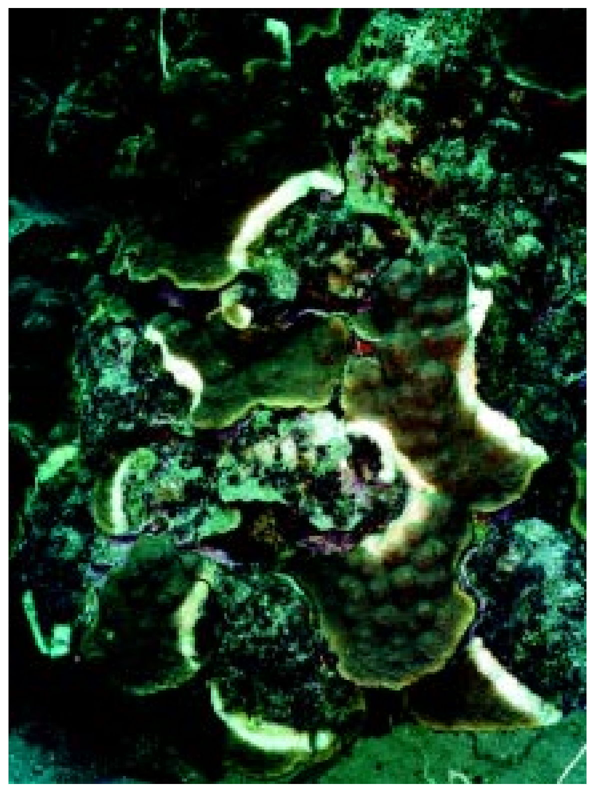


EXECUTIVE SUMMARY

The health and continued existence of coral reef ecosystems are threatened by an increasing array of environmental and anthropogenic impacts. These causes of decline, including global climate change, invasive species, shoreline development, habitat destruction, polluted runoff, sedimentation and over-exploitation overwhelm the natural plasticity of these systems and have contributed to an estimated loss of 27% of the world's reefs. If current pressure continues unabated, nearly 60% of the world's reefs may be lost by 2030, due to reduced coral growth rates, bleaching, disease outbreaks and increased mortality. These ecosystems, which are among the most complex and biologically diverse habitats on earth, provide economic and environmental services to millions of people worldwide. In order to preserve and protect these ancient marine ecosystems, an understanding of the effects of natural and anthropogenic stressors on reef-building coral communities is essential.

Coral diseases have not been thoroughly characterized and etiologies, including many of the causative agents remain uncertain. In an effort to form a cohesive national strategy to conserve and protect these ecosystems, the federal government was directed to strengthen its stewardship of the nation's reef ecosystems and coral reefs around the world. This executive order, issued by President William Jefferson Clinton on June 11, 1998, resulted in the establishment of the *United States Coral Reef Task Force* (CRTF). A collective response was produced by this Task Force in the form of a *National Action Plan to Conserve Coral Reefs*, which represents the road map for U.S. coral reef conservation efforts. This was followed by a National Coral Reef Action Strategy, which is a report to Congress that fulfills the requirements of the Coral Reef Conservation Act of 2000 and helps track implementation of the National Action Plan. Conservation actions of this plan and strategy encompassed two fundamental themes: an understanding of coral reef ecosystems and a reduction of the adverse impacts of human activities. To fill a gap in our understanding of the rapidly emerging threats to reefs worldwide, the CRTF's Working Group on Ecosystem Science and Conservation recommended the formation of a Coral Disease and Health Consortium (CDHC). The primary objectives of the CDHC are to organize and coordinate scientific resources nationally and internationally to address coral health issues, with emphasis on the diagnosis, etiology and epizootiology of coral diseases and bleaching.

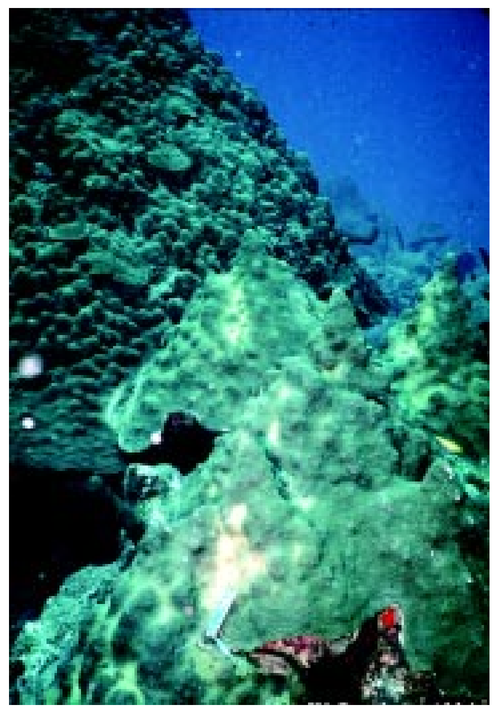


Many of the emerging coral diseases have not been fully characterized such as yellow-blotch disease (YBD), shown here. Outbreaks of YBD have been reported from several Caribbean localities, primarily affecting star corals in the genus *Montastraea;* little is known about causes or long-term impact. *(Photo: Andy Bruckner).*

The first official meeting of the CDHC was convened in Charleston, South Carolina, from January 22-25, 2002. This workshop brought scientists, resource managers, and regulatory officials together to identify gaps in our understanding of the causes of the worldwide coral reef decline; provide a coordinated focal point for research on and tracking of the occurrence and progression of bleaching and disease events; and help identify environmental influences that affect these phenomena. A number of nationally recognized experts in the areas of biology, ecology, pathology, coral disease, biochemistry, molecular biology, cellular physiology, environmental microbiology, human and veterinary medicine and biotechnology were selected to represent critical scientific disciplines. Position papers, on 10 relevant topics and written by participants, were distributed and formed the basis for discussion within four working groups. The working groups were organized around four themes: *Biology*, *Environmental Factors Affecting Infectivity and Susceptibility*, *Disease Identification and Disease Investigation*, and *Diagnostics*. Participants were divided among the working groups according to their areas of expertise. Roundtable discussions within groups were convened over a three-day period. Conclusions of the working groups were presented to all participants in one plenary session.

Plenary session at the first CDHC Workshop in Charleston, SC. *(Photo: Darren Wray)*.

Regional and global bleaching events have increased in frequency since the mid 1980s with widespread mortality observed with the 1998 bleaching event. Bleaching has been correlated with a wide range of environmental and physiological stresses, but its physiological mechanisms are not fully understood. Bleached colony of *Montastraea faveolata* (left) regains most of its pigmentation (right) six months later. *(Photos: Andy Bruckner)*.



Many strategic research objectives were identified. The major objectives can be summarized as follows:

Establishment of standard terminology, methodology and protocols: There is limited application of human and veterinary medical knowledge to the study of coral health and disease; standard medical terminology has not been used in descriptive interpretations of clinical anomalies noted during field collections or in the laboratory, resulting in ambiguous and often misleading communication of findings. This is compounded by inadequate diagnostic tools and a deficiency in consistent, comprehensive monitoring, assessment and collection protocols. A review of the existing and emerging coral disease syndromes that institutes clinical diagnostic criteria, including visual and descriptive details is paramount to understanding and combating coral disease.

Expansion of knowledge in basic coral physiology, biology and disease etiology: There is limited information on the physiological parameters that define healthy coral and even less on coral disease dynamics. By expanding our understanding in areas of cellular physiology, genomics and proteomics we will be able to (1) define baseline measures of coral health and vitality such as non-stress levels and deviations that indicate an altered state; (2) identify normal changes in physiological parameters occurring seasonally, annually, and along geographic, bathymetric, and other physical and/or environmental gradients to increase diagnostic accuracy; (3) identify mechanisms of resistance and susceptibility to disease and factors that modulate defense mechanisms; and (4) characterize processes at individual, population and community levels that affect ecological connectivity of reefs and discover critical control points for management strategies.

Development of model coral species: All aspects of coral disease and health research have been hampered by the lack of an established coral laboratory model species (analogous to "lab rats"), coral cell lines and standardized sources of algal and bacterial cultures that are routinely available for research. There is a critical need to develop models and provide the infrastructure to maintain these living stock collections. Providing well-characterized and documented experimental organisms to domestic and international researchers will enable rapid advances through the use of modern biomedical techniques to investigate coral health and disease and by focusing research on fundamental biological concepts broadly applicable across the taxon.

Development of a Centralized Data/Knowledge System, Website, Repository and Core Diagnostic Facilities: Understanding mechanisms of coral disease in order to devise appropriate treatment, mitigation or management regimes has been limited by static databases, comprised of independent datasets that are incompatible with modern integrated analyses. New analytical technologies of bioinformatics now allow the development of an "intelligent" system that can assimilate data of all types into information that can be synthesized into knowledge. Application of bioinformatics to the study and investigation of coral health and disease is critically needed to disentangle the multi-factorial nature of coral disease dynamics. The logistics of this approach demands a centralized system that receives data from field units and is supported by regional response teams, diagnostic laboratories with critical assay capabilities and an underpinning of training and voucher specimen archive.

VISION: ***"To understand and address the effects of natural and anthropogenic stressors on corals in order to contribute to the preservation and protection of coral reef ecosystems."***

Recommendations to address strategic research were proposed during working group deliberations and various post workshop committees were established. Recommendations, too numerous to list in this summary, and committee tasks are described in detail in this report. They are also embodied in the following Vision and Goals statements (non-prioritized) adopted by the Coral Disease and Health Consortium.

The primary deliverable of the CDHC workshop was to develop a Strategic National Research Plan directed towards the study of coral disease and health that highlights the following SPECIFIC GOALS:

- Establish targeted committees of experts to review, assess, develop, organize and implement coral disease and health needs and approaches highlighted in the CDHC National Research Plan.

- Establish and promote standardization of coral disease and health research through development of common terminology, interpretations, monitoring protocols, collection techniques, reporting standards, and laboratory protocols.

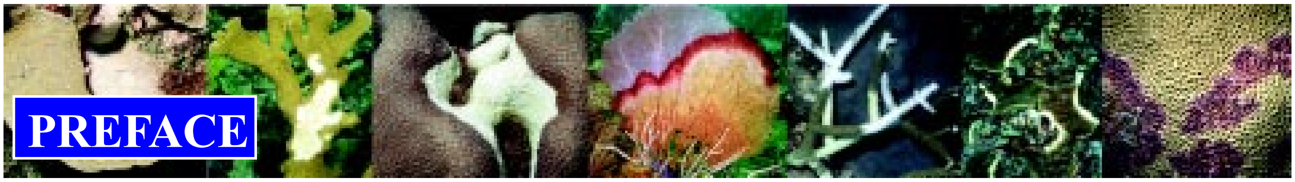
- Determine the occurrence, causes and effects of declines in coral health.

- Define effects of point and non-point discharges and environmental and climatic stressors on coral health through understanding mechanisms of action.

- Provide technical information and practical diagnostic tools to help managers and scientists understand, evaluate, track, predict and remedy coral diseases.

- Strengthen and coordinate opportunities for multidisciplinary collaborations and provide cross disciplinary training for scientists and managers.

Students receiving training in laboratory techniques for the study of coral disease at a Molecular Techniques workshop in Hawaii. *(Photo: Teresa Lewis).*



PREFACE

Decline in live cover, species richness, and condition of reef building corals is rising in frequency and distribution worldwide. This is most apparent through reduced coral reef growth rates, bleaching, disease outbreaks and increased mortality. A growing number of coral species and associated coral reef organisms have been affected. Cause and effect relationships have not been well documented and new disease syndromes continue to emerge. Presumably, emerging diseases are caused by the interaction of biotic and abiotic stressors, however, coral health and diseases have not been thoroughly characterized and disease etiologies, including the causative agents, remain uncertain. The nature of this problem has changed considerably over the last two decades and today's research, monitoring, and regulatory infrastructure are not adequately prepared to meet the expanding threat.

Over the last few years, there has been a major expansion in international efforts to monitor the world's coral reefs and promote sound coral reef conservation strategies through various international and regional fora including the International Coral Reef Initiative (ICRI), Convention on International Trade in Endangered Species of Fauna and Flora (CITES), Convention on Biological Diversity (CBD), Ramsar Convention on Wetlands, and the Asia Pacific Economic Cooperative (APEC). These efforts began in 1994, with the formation of ICRI and establishment of its major operational unit, the Global Coral Reef Monitoring Network (GCRMN). The GCRMN currently has representatives from 86 countries or states, and has issued reviews of the status of the world's reefs every two years since 1998 (Wilkinson C (ed), 1998). One of the primary sources of information on the global health of coral reefs used in these reports has come from Reef Check, a program based at the University of California involving an international network of regional, national and local volunteers that monitor coral reefs in over 50 countries. These and other data are incorporated into Reefbase, an ongoing effort of assembling available knowledge about coral reefs into one information repository.

In June 1998, President Clinton introduced Executive Order 13089, urging all arms of the US Government to do their utmost to map, document, research and conserve the coral reefs under US jurisdiction, as well as assist and collaborate with international agencies, partners and governments including ICRI and the GCRMN to promote conservation and sustainable use of coral reef resources worldwide. This order established the US Coral Reef Task Force (CRTF), chaired by the Secretary of the Interior and the Secretary of Commerce and comprised of many federal, state and territorial government agencies, whose primary responsibilities were to oversee implementation of policy and Federal agency responsibilities to preserve and protect the biodiversity, health, heritage, and social and economic value of U.S. coral reef ecosystems and the marine environment. More recent efforts include, the International Coral Reef Information Network (ICRIN), established in 1999, to raise awareness about coral reefs and particularly targeted senior decision makers. The U.S. government, through NOAA, has created the Coral Reef Information System (CORIS), an internet-based repository for U.S. data on coral reefs. In addition, through the Coral Reef Conservation Act of 2000 Congress has mandated the development of a biennial report on The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States, with the first report issued in 2002 (Turgeon *et al*., 2002).

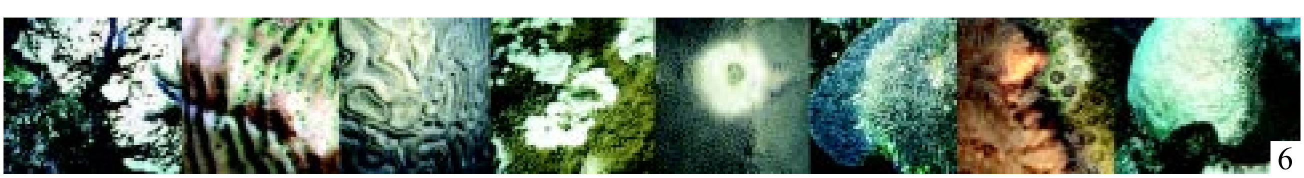
One of the key findings of most, if not all monitoring programs, is that coral reefs are declining globally, and coral diseases and bleaching are recognized as one of the key threats. In an effort to combat expanding threats to our coral reef ecosystems, the Coral Disease and Health Consortium (CDHC) was formed at the recommendation of the US CRTF (2000) to organize and coordinate scientific resources and address ecosystem-scale threats impacting stony corals and soft corals. The Consortium is being implemented through interagency partnerships of NOAA, EPA and DOI, with involvement by over 35 partner institutions, both domestic and international. The main objective of the CDHC was to focus on the issues surrounding coral health and disease. In January of 2002, the CDHC held its first organizational workshop to address relevant issues of coral health and disease in the context of coral reef biology and ecology. Participants represented critical scientific disciplines and many geographic regions. These individuals were selected from the ranks of academia, state and public health services, the biotechnology industry, non-profit institutions, as well as several US government agencies: The Department of Interior (DOI), the Environmental Protection Agency (EPA), and the National Oceanic and Atmospheric Administration (NOAA). Attendance was limited to key experts to promote close working relationships during the workshop.

Several workshop participants were assigned topics to review within their areas of expertise and requested to prepare a position paper. The position papers were to address background, current state of knowledge, impediments limiting progress, and prioritized research topics. Position papers were distributed to all participants prior to the workshop.

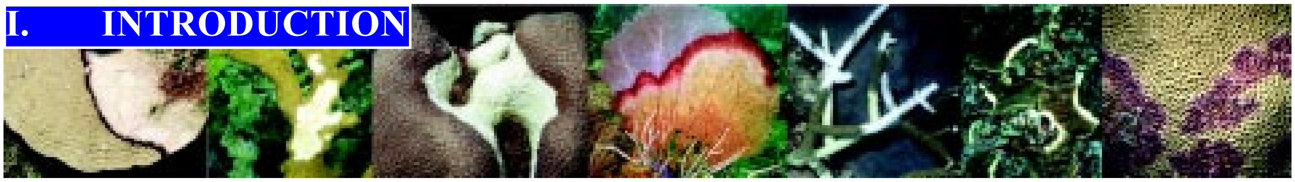
Four working groups formed the basis of the workshop: *Biology*, *Disease Investigation and Disease Identification*, *Environmental Factors Affecting Infectivity and Susceptibility*, and *Diagnostics*. During individual working group deliberations, lists of information gaps, issue driven research objectives and recommendations were prepared. These lists were presented to all participants in a plenary session at the end of the four day workshop.

Given this procedure and the related nature of the issues discussed by the different working groups, it is not surprising that some issues appear several times in the lists of research objectives and recommendations that follow. Rather than arbitrarily removing these common issues from subsequent sections after their first mention, the overlapping issues were highlighted in each group and left unchanged to emphasize the cross-disciplinary importance of certain issues.

There was considerable discussion on the need to prioritize the recommendations. The attempt to do this in some of the working groups was not successful, as most participants felt that the list of recommendations had already been distilled from a much larger list and thus reflected priority issues. Another concern was that it was difficult to establish absolute priorities between very different topics since such decisions would vary dramatically among individuals or agencies with different responsibilities or interests, and priorities also vary among coral reefs in different regions. Accordingly, the recommendations in this Research Plan are not prioritized. They are grouped by topic, so that agencies developing forecasting, monitoring and assessment, or research programs can work from the lists that most closely match their purview. All are deemed of high (and equal) priority, though it should be recognized that several recommendations appear in each of the four topic areas and thus have general applicability across all disciplines.



I. INTRODUCTION



Coral reefs are the most complex and biologically diverse marine ecosystems on the planet. These systems provide economic and environmental services to millions of people as areas of natural beauty and recreation, sources of food, jobs, chemicals, pharmaceuticals, and shoreline protection. Today, the world's coral reefs are continually threatened by an increasing array of stressors including over-exploitation, pollution, habitat destruction, invasive species, disease, bleaching and global climate change.

Worldwide, coral reefs are found in over 100 countries and cover an estimated 284,300 square kilometers (ICRIN, 2002). Global climate change as well as the direct and indirect effects of anthropogenic stressors have contributed greatly to the degradation and loss of an estimated 27 percent of the world's coral reefs and a much greater percentage is under increasing threats. Coral reefs are a crucial life support system of the biosphere and may be the first major marine ecosystem to show significant impacts from global climate change. In recent years, there has been progress in implementing global monitoring networks and providing sound information on the status of the world's reefs. However, there are large regions of the world where data are insufficient to determine the condition of many coral reefs, as well as locations for which no baseline assessments exist. Research teams have made some progress in investigating coral disease and documenting the distribution of certain outbreaks, although these efforts have largely targeted areas in the wider Caribbean near human population centers. Despite these efforts we remain uncertain of the complex mechanisms underlying the increases in coral bleaching events, disease outbreaks and perhaps most disturbing, the influence of human activities on these processes. Also many of the technologies and tools needed for an efficient and accurate health assessment of these systems await development or adaptation from existing medical knowledge.

Tiger grouper and coral (*Montastraea cavernosa, Eusmilia fastigata and Psuedoterigorgia* sp.). *(Photo: Andy Bruckner).*

Our lack of understanding of the underlying mechanisms of coral pathologies inhibits the ability to manage these growing coral health problems as reflected in the major bleaching events documented in 1997-98, early 2000 and in 2001, and in increases in disease incidence and prevalence (Porter and Porter, 2002; Hoegh-Guldberg, 1999). The United States Executive Order 13089 of 1998 brought local emphasis to the global problem and manifested a growing awareness that more of our national resources must be focused on coral reef preservation. Sound input is urgently needed from scientists, industry, resource managers, and regulatory officials to keep these new research initiatives focused on high priority, productive endeavors that contribute to a better understanding of these pathologies and to the development of management measures to mitigate their impacts. Therefore, the primary goal of this workshop was to formulate a National Research Plan consisting of a series of recommendations intended to address the major gaps in the research arena of Coral Disease and Health.

The Workshop participants formulated the following overall Vision and Goals for a *National Research Plan on Coral Disease and Health:*

VISION:
"To understand and address the effects of natural and anthropogenic stressors on corals in order to contribute to the preservation and protection of coral reef ecosystems."

Elkhorn coral (*Acropora palmata)* thicket on Mona Island, Puerto Rico. *(Photo: Andy Bruckner).*

SPECIFIC OBJECTIVES:

- Establish targeted committees of experts to review, assess, develop, organize and implement coral disease and health needs and approaches highlighted in the CDHC *National Research Plan*.
- Establish and promote standardization of coral disease and health research through development of common terminology, interpretations, monitoring protocols, collection techniques, reporting standards, and laboratory protocols.
- Determine the occurrence, causes and effects of declines in coral health.
- Define effects of point and non-point discharges and environmental and climatic stressors on coral health through understanding mechanisms of action.
- Provide technical information and practical diagnostic tools to help managers and scientists understand, evaluate, track, predict, and remedy coral diseases.
- Strengthen and coordinate opportunities for multidisciplinary collaborations and provide cross disciplinary training for scientists and managers.

II. BIOLOGY

1. Background

Coral reefs and their associated sea grass, algae and mangrove habitats are among the most diverse and valuable ecosystems on earth. Such reef systems are storehouses of immense biological wealth and provide economic and environmental services to millions.

Fishers and community members in Priory, Jamaica are retrieving the days catch *(Photo: Andy Bruckner).*

The 2002 NOAA report on the state of coral reef ecosystems calculated an annual 45 million visitors to the US coral reefs, generating an estimated $17.5 billion annually (Turgeon et al., 2002). The global estimate of the value of coral reefs to the world economy in 1997 was $US375 billion in economic and environmental services, including sources of food, pharmaceuticals, employment, coastal protection, recreation and tourism (Costanza et al., 1997). However, the investment in research, monitoring and management is less than 0.05% of that value (Wilkinson, 2002).

Coral reefs are massive calcium carbonate structures that result from biomineralization by anthozoan animals (corals) and coralline algae. Although small in cumulative area (< 0.2% of the ocean floor), coral reefs are among the world's most diverse ecosystems, supporting >25,000 described species and possibly another 1-8 million undescribed species (Connell, 1978; Birkland, 1997; Dustan 1999; Koop et al., 2001; Reaka-Kudla, 1996). These represent 32 of the 34 animal phyla, including a rich invertebrate and fish fauna (Karlson and Cornell, 1998, 1999). In addition, coral reefs protect shorelines from erosion, and shelter other associated ecosystems (i.e., mangroves, sea grass beds) that are important nursery grounds for commercially and recreationally important fishes and invertebrates. Coral reefs are critical habitat for over 20% of the world's fisheries and over 50% of all US federally managed fisheries species, providing much of the protein consumed by human populations in coastal tropical zones (Hoegh-Guldberg 1999).

Many species of grouper are commercially harvested from coral reefs worldwide, including this Nassau grouper from the Caribbean. *(Photo: Andy Bruckner).*

Over the past three decades, coral reefs have experienced extensive degradation worldwide, with both anthropogenic and non-anthropogenic forces implicated in declining coral reef health. Anthropogenic stresses include global warming, changes in salinity and turbidity, and agricultural, suburban, and industrial discharges, as well as increasing pressure associated with unsustainable extraction of corals and coral reef species for food, building materials, pharmaceuticals, curios and other uses. Non-anthropogenic factors include biological agents such as predation, competition from algae and other encrusting organisms, and diseases, which may be exacerbated by anthropogenic effects (Richmond, 1993; Bryant and Burke, 1998; Wilkinson, 1999; 2000, 2002; Turgeon et al., 2002). Despite the important ecosystem services they provide and their critical role sustaining marine biodiversity in shallow tropical waters, relatively little is known about many basic aspects of coral biology. This presents major challenges in identifying, understanding, and ultimately controlling coral diseases.

2. Challenges and Recommendations

The Biology Working Group (BWG) identified six strategic objectives as crucial to understanding coral health and disease. These objectives emerged during roundtable discussions, and all participants agreed that the best strategy for pursuing them was through peer-reviewed, competitive grants unrestricted either geographically or taxonomically. The BWG recognized that some goals could be achieved quickly (given adequate funding) while others require more substantial commitment. Ideally, investigations should enhance collaborative research and infuse coral biology with new techniques and ideas, including those from areas not traditionally considered "marine biology." Mechanisms to achieve this include interdisciplinary graduate and post-doctoral fellowships, and training grants. To translate research findings into management strategies, the BWG advocates close collaboration between scientists and resource managers, publishing results in peer-reviewed journals, educating the public, and including all parties in problem-solving.

One of the main challenges highlighted by the BWG is the availability of funding to conduct the necessary research and education. The group recommends that funding research on coral diseases and the establishment of supply facilities for research organisms through merit-based competitive grants and cooperative agreements should be encouraged. One way to achieve this goal is through targeted RFPs from The National Science Foundation (NSF) and/or other cognizant agencies (Environmental Protection Agency [EPA], National Oceanic and Atmospheric Administration [NOAA], and the Department of the Interior [DOI]) for the development of an international collection of live corals as research model organisms along with techniques to culture and maintain specific coral species for use in laboratory studies as well as coral and zooxanthellae cell cultures. For example, NSF's Division of Biological Infrastructure supports Living Stock Collections and the development and maintenance of repositories of research organisms, genetic stocks and cell lines, as well as DNA clones associated with whole organisms in a collection. NSF may also provide funds for curatorial databases, and for linking the information associated with the collection to other information resources and scientific databases, such as the CDHC database. A portion of federal, state and NGO funding supporting coral reef efforts should be directed towards the establishment of supply facilities and the development and maintenance of specific databases, repositories and websites that compile and link information resources and scientific databases that will synthesize information through advanced computational analyses of bioinformatics and systems biology. In addition, new funding is necessary for these activities as well as specific research, monitoring, forecasting, and assessment programs geared towards understanding and addressing the underlying cellular physiological mechanisms controlling coral health. These resources are considered essential for the support of national and international scientific research in the biological sciences and specifically for the development of coral and zooxanthellae culturing facilities.

STRATEGIC OBJECTIVE 1: Determine the mechanism(s) of coral bleaching.

RECOMMENDATION: Conduct research on the cellular and molecular mechanisms of coral bleaching.

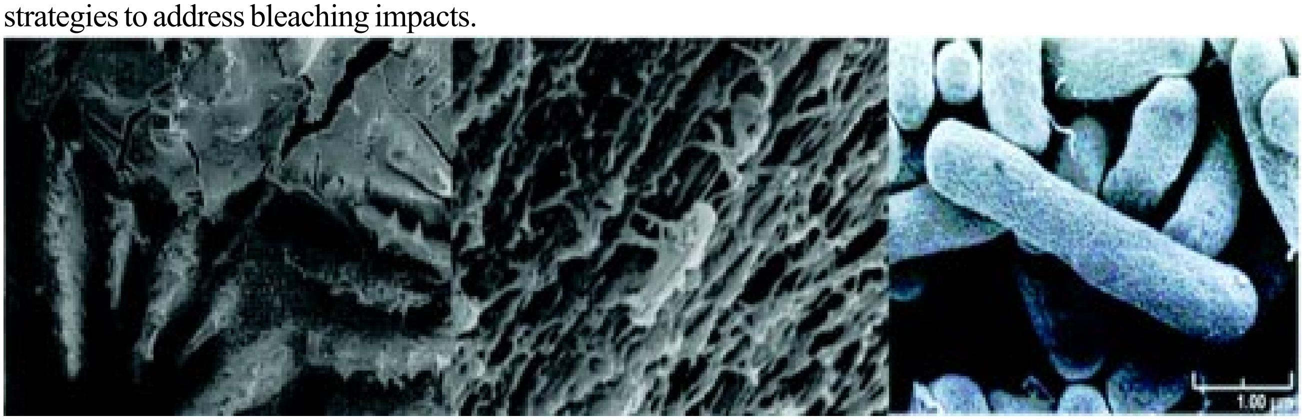
In 1998, there was a major paradigm shift among coral reef scientists and managers regarding major causes of the degradation of reefs. While direct anthropogenic impacts of nutrient and sediment pollution and overfishing were previously identified as the major threats to reefs, global climate change was identified as a new and comparable threat due to major switches in climate, warming surface waters and increases in CO_2 concentrations responsible for massive coral "bleaching" and mortality over large areas of reefs worldwide. Bleaching, which occurs when corals lose their algal (dinoflagellate) symbiont or the symbionts' photosynthetic pigments degrade, can be caused by a wide range of environmental and physiological stresses, such as heat, cold, solar radiation, pollution, reduced salinity, changes in oxygen concentration and microbial pathogens. In the laboratory, bleaching is induced by diverse factors, including elevated water temperature, cold temperature, supersaturating light and bacterial infection. However, most models of coral bleaching are phenomenological, and do not specify the cellular and molecular mechanisms required to understand the degradative process, or to identify what causes bleaching on individual reefs.

Currently, there are two popular, competing models of coral bleaching, the oxidative stress model and the pathogen model:

1. The oxidative stress model postulates that elevated temperature destabilizes the electron-transport chain of the photosynthetic algae, causing increased production of reactive oxygen species. As a last defense against these damaging molecules, the coral expels its algal symbionts.

2. The pathogen model proposes bleaching is caused by opportunistic infections by the bacterium *Vibrio*. This model postulates high temperature lowers coral resistance to infection and/or increases bacterial virulence, perhaps by enhancing its ability to adhere to the coral.

Testing these and other possible models of coral bleaching to determine the prevalence, relative importance, and geographic amplitude of the mechanisms of coral bleaching is vital for resolving whether bleaching events ascribed to global warming actually are caused or accelerated by opportunistic infections and to develop strategies to address bleaching impacts.



Controlled laboratory experiments highlight the ability of certain bacteria to become infectious, meeting Koch's postulates. Photomicrographs above show diseased coral skeleton (left), bacteria on diseased/denuded skeleton (middle), and the putative disease agent (right). *(Photos: Shawn Polson)*.

STRATEGIC OBJECTIVE 2: Develop model laboratory species (i.e., the coral equivalent of a "lab rat") to spur advances in coral research.

RECOMMENDATION: Identify and develop model species that are well characterized and easy to culture, and create a facility to culture, maintain and supply model species to scientists.

To investigate normal coral biology and disease states using modern scientific techniques, it is necessary to identify and develop model species, and make them routinely available for research. Model laboratory species share well-known desirable characteristics, including ease of culture, high growth and fecundity rates, and relatively simple genetics. Model corals will be analogous to "lab rats," and enable rapid advances by focusing research on fundamental biological concepts broadly applicable across the taxon. Model corals must be representative of coral diversity, and include IndoPacific and Caribbean species, autotrophs and heterotrophs, branching and boulder growth forms, species with different calcification rates, and with different algal symbionts. They also must include taxa susceptible to bleaching and disease and resistant taxa. Developing a living stock collection for model corals will provide infrastructure critical for basic research by providing well-characterized and documented experimental organisms to domestic and international researchers at a modest charge (the Indiana University Axolotl Colony [http://www.indiana.edu/~axolotl/] is a working model of such a facility). The BWG recommends developing model species and establishing supply facilities through merit-based, peer-reviewed grants.

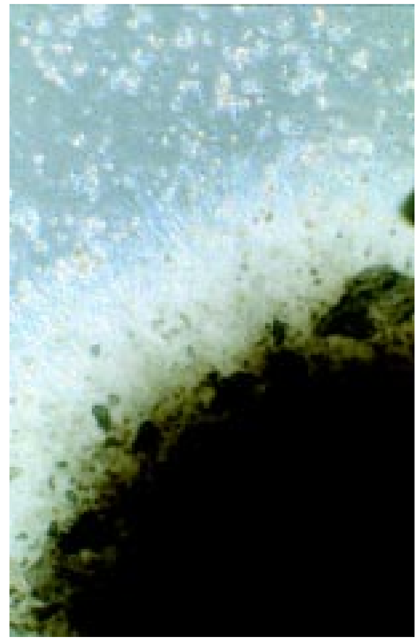
Laboratory culturing of *Acropora cervicornis.* Under the direction of Dr. Erich Mueller, Mote Marine Laboratory, Center for Tropical Research in the Florida Keys has been propagating several coral species. The ability to propagate and culture corals of known genotypes is the first step toward developing laboratory model species for scientific research and conservation efforts. *(Photo: John Bemiss)*

Species that are resistant to diseases and have varying life histories are also cultured for research models, such as *Porites astreoides*, which broods larvae (left) or the Pacific pocilloporid, *Pocillopora damicornis* (right). *(Photos: Andy Bruckner).*

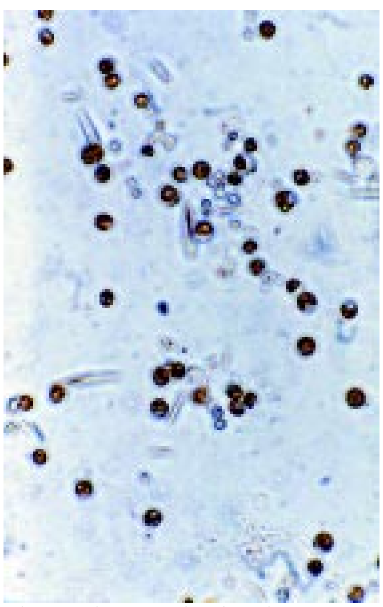
STRATEGIC OBJECTIVE 3: Establish coral and zooxanthellae cell cultures for use in laboratory research.

RECOMMENDATION: Determine the special requirements needed to establish and maintain coral and zooxanthellae cell cultures, and establish facilities that can maintain these cultures and supply them to scientists.

Developing short- and long-term primary coral cultures in addition to immortalized cell lines as an alternative to animal experimentation is essential to laboratory studies of normal and disease states, including gene sequencing and expression. It also is essential for augmenting dwindling native stocks of rare corals for use in research. Currently, there are only a few investigators able to establish primary coral cultures but no immortalized coral cell lines are available in such repositories as the American Type Culture Collection (ATCC). Culture collections of the coral's symbiotic algae are limited to a few private collections. Determining the special requirements needed to establish and maintain coral and zooxanthellae cell cultures will enable researchers to use modern biomedical techniques to investigate coral health and disease. Once cell lines are successfully established and maintained, they should be incorporated in the ATCC, where they are available to researchers for a nominal fee. One approach involves encouraging facilities that maintain living cultures of algae (i.e., Provasoli-Guillard National Center for Culture of Marine Phytoplankton) to add zooxanthellae to their collection.



Explant culture of *P. damicornis* coral cells. *(Photo: Gary Ostrander).*



Phase micrograph of various types of coral cells in primary culture after 48 hours. *(Photo: Gary Ostrander).*

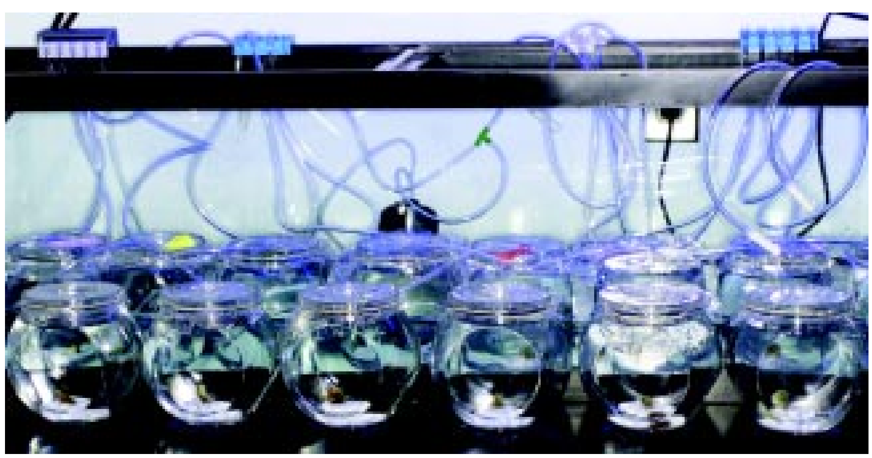
Symbiodinium cultures. The symbiotic algae from coral is being grown in large quantities for laboratory studies. Cultures provided by Drs. Todd La Jeunesse and Bill Fitt. *(Photo: Cheryl Woodley).*

STRATEGIC OBJECTIVE 4: Determine physiological parameters typical of normal, healthy coral.

RECOMMENDATION: Determine boundaries of normal variation in coral health, including ontogenetic, temporal, bathymetric, geographic, and species-specific responses.

Coral physiology is poorly understood and quantified, making it difficult to distinguish disease states from natural variations in condition. Therefore, it is vital to determine baseline measures of coral health and vitality at the genetic, molecular, cellular, tissue and whole organism levels. Determining nominal (non-stressed) levels of relevant parameters requires reference values from healthy individuals, as inferred from both laboratory and field studies. Deviations from the nominal range then indicate an altered state, which is defined as diseased, if it is associated with a deteriorating condition that ultimately reduces individual performance (i.e., growth, reproduction, immunological competence, etc.). Therefore, investigations must quantify what is normal in representative coral species (preferably in the "model species"), and determine how physiological parameters and fitness components change during ontogeny, seasonally, annually, and along geographic, bathymetric, temperature, and other clinal gradients. Once nominal values are defined, investigators can more readily identify disease states, diagnose likely causal agents, and forecast the ecological and economic consequences of epizootics. Achieving this goal requires supporting long-term research, and multi-investigator teams working at multiple levels of biological organization. The BWG recommends vertically integrating field studies to take advantage of ongoing monitoring projects, and emphasizes the importance of experimental studies, both in the laboratory and on coral reefs.

Laboratory maintained *Oculina varicosa* receive regular feeding of *Artemia* larvae. *(Photo: Dorothy Howard).*



Laboratory experiments are critical to understanding how corals respond to individual stressors and combinations of stressors. Such experiments give us controlled conditions to explore the effects of both biotic (i.e., disease pathogen) and abiotic (i.e., xenobiotic) stressors. Here *Oculina varicosa* fragments, supported on plastic platforms in individual dosing chambers, are being challenged with a putative bleaching pathogen. *(Photo: Darren Wray).*

STRATEGIC OBJECTIVE 5: Determine the basic host defense mechanisms and immune responses of corals.

RECOMMENDATION: Conduct basic research on coral defense mechanisms and immune system.

Despite increased attention to coral bleaching and disease outbreaks, little is known about host defense mechanisms. Coral mucus serves a protective role, and is active in the removal, lysis, and consumption of bacteria. Both the coral and its symbiotic algae produce amino acids that act as natural sunscreens, and can regulate enzymatic activity to remove noxious oxygen radicals produced by heat and light stress. Comparatively little is known about coral immune systems; researchers suspect lipopolysaccharides and glucan-binding proteins are involved with host defense and that corals have lytic systems, and surface-recognition systems ancestral to those commonly found in innate immunity. Beyond these generalities, extremely little is known about coral host defense mechanisms and immunology. Bridging this data gap is a major challenge for the Coral Disease and Health Consortium. Fortunately, considerable expertise already exists in biological sub-disciplines outside the realm of coral biology. Therefore, the BWG recommends encouraging interdisciplinary research by funding proof-of-concept and training grants, and dissertation and postdoctoral fellowships to attract immunologists and biochemists to conduct basic research on defense mechanisms and immune systems in corals, sea fans, and their relatives.

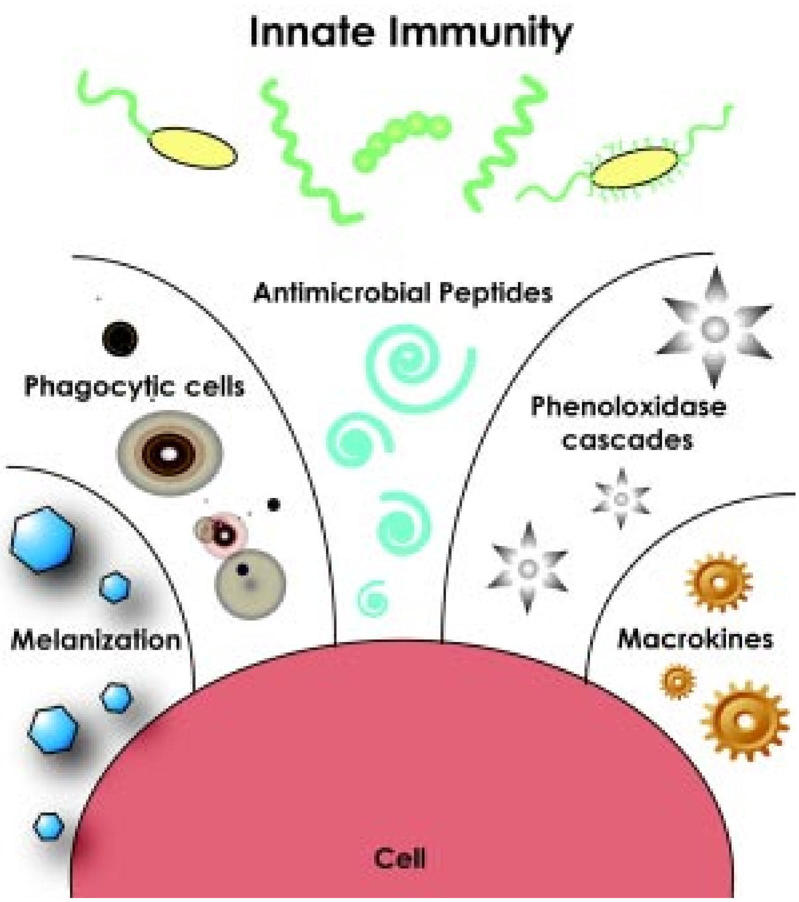
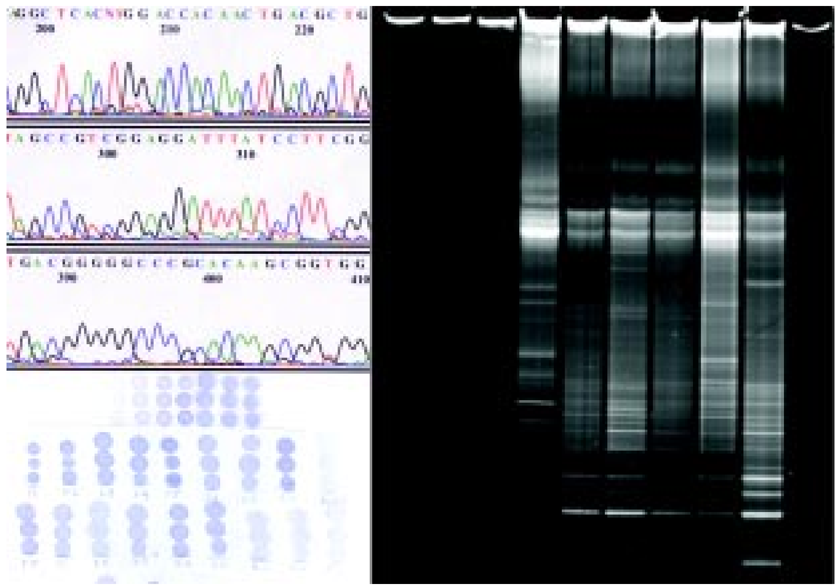


Diagram of components associated with innate immune systems. Corals like other invertebrates possess an innate immune system. This system is characterized by its ability to rapidly respond to microorganisms because its detection mechanism is based on recognizing surface structures that are common among large groups of microbes. *(Artwork: Sara Polson).*

STRATEGIC OBJECTIVE 6: Determine patterns of coral gene expression, and genetic mechanisms of resistance and susceptibility to disease.

RECOMMENDATION: Sequence the genomes of representative coral species and their symbiotic dinoflagellates.

Sequencing the genome of a representative coral species and their symbiotic dinoflagellate algae will allow researchers to interpret gene function in healthy and diseased colonies. The objective is to identify all the genes in coral DNA, determine their sequences, store the information in accessible databases, and compare them with reference DNA sequences in organisms that are better understood (i.e., human, mouse, fruit fly) to understand gene function. Benefits include identifying genes expressed in normal versus stressed or diseased individuals, including genes that increase susceptibility or confer resistance to bleaching and disease. Recent technological advances in gene sequencing and bioinformatics, coupled with the relatively small size (1.12 x 10^9 bp/haploid genome vs 3.45 x 10^9 bp/haploid genome of humans) of many coral genomes (ca. 28 pairs of chromosomes) permit this goal to be accomplished quickly once funding is secured and research efforts are coordinated. The symbiotic dinoflagellates, however, have genome sizes estimated to be 100X larger than the coral genome. Thus new strategies will likely need to be employed in order to sequence the dinoflagellate genome.



Molecular diagnostic techniques used to study coral disease: DNA sequencing chromatogram (upper left), protein concentration assay (lower left) and DGGE (denaturing gradient gel electrophoresis) (right). *(Figures: Sara Polson).*

Biology Working Group Members (BWG)

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Mohamed Faisal – Michigan State University, Living Oceans Foundation
John Fauth (Chair) - College of Charleston
Sylvia Galloway – NOAA/National Ocean Service
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Jeff Miller - Virgin Islands National Park
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James Porter - University of Georgia
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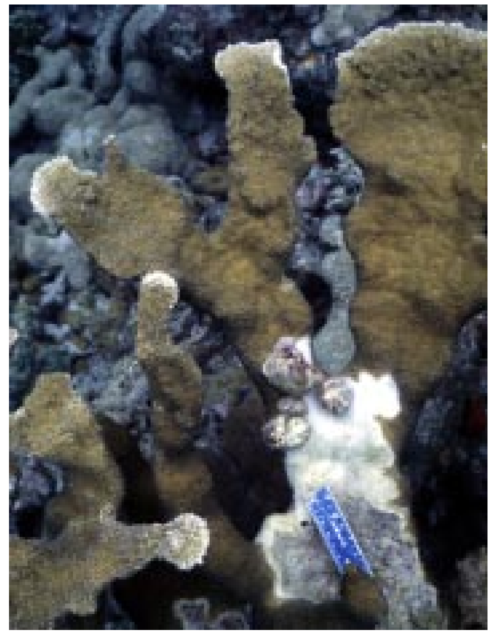
II. DISEASE IDENTIFICATION AND DISEASE INVESTIGATION

1. Background

"Disease" is defined by the CDHC as *any deviation from or interruption of the normal structure or function of any body part, organ, or system, which is manifested by a characteristic set of signs and whose etiology, pathology and prognosis may be known or unknown.*

Emerging coral disease causation has been linked to biotic and abiotic stressors and to their synergistic interactions. However, traditional coral health assessment has only been able to detect effects after degradation or disease outbreaks have occurred, thus providing only indirect measures of stressor effects. Although over 35 different coral diseases and syndromes have been reported worldwide (Green and Bruckner, 2000), only a limited number of these have been verified through the peer-reviewed literature (Richardson, 1998). The majority of described coral diseases have not been thoroughly characterized, most pathogens associated with these diseases are unknown, and the mechanisms and pathways involved in transmission of a pathogen, infection and subsequent mortality are poorly understood. In the western Atlantic, only black-band disease (BBD), white plague, yellow-blotch disease, white-band disease (WBD) and neoplasia are readily identifiable in the field, while other conditions such as dark-spots disease, white pox, red-band disease (RBD) and various "types" of diseases (e.g., plague type II and III; WBD type II) can be easily misidentified as another disease or syndrome, or can be confused with signs of predation or other causes.

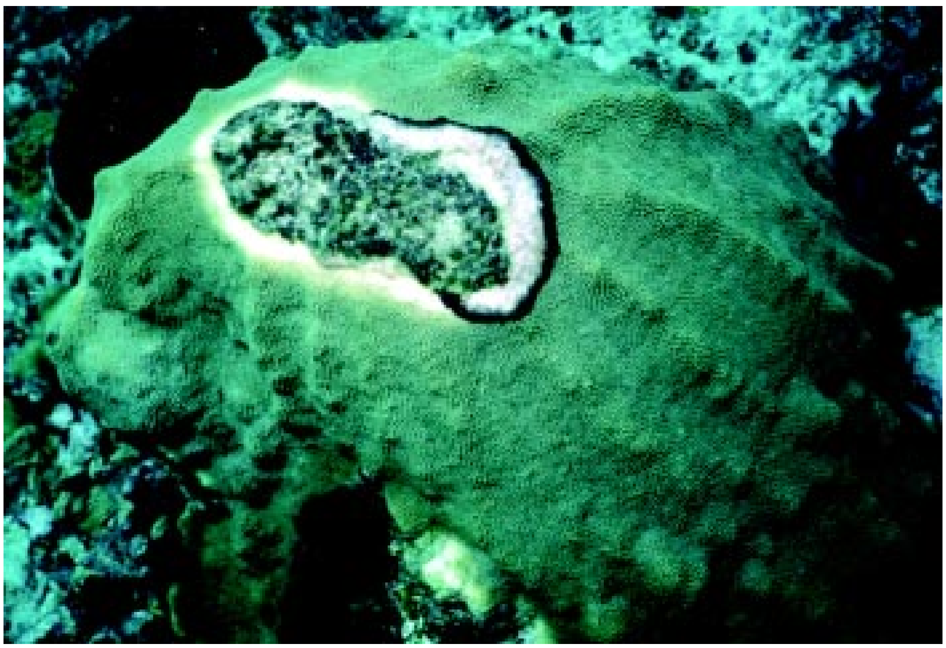


Coral diseases can be easily confused with other causes of mortality. For instance, predation by *Coralliophila abbreviata* snails (shown above) may be misidentified as white-band disease (shown below) if the snails are on the underside or base of the colony. *(Photos: Andy Bruckner).*

Furthermore, the presumed pathogens have only been verified to meet Koch's postulate for plague type II (*Aurantimonas coralicida, gen nov. sp. nov.,* Denner et al., 2003; Richardson *et al.,* 1998*)*, Aspergillosis (sea fan disease) (Smith et al., 1996), white pox (*Serratia marcescens,* Patterson et al., 2002, and bleaching (*Vibrio* spp., Kushmaro et al., 1996; 1997; Rosenberg and Ben-Haim, 2002). In other diseases such as BBD and RBD, a consortium consisting of various bacteria, cyanobacteria, fungi and/or other microorganisms have been found associated with infected colonies, but these microorganisms have not been demonstrated to be the causative agents through vigorous laboratory studies that fulfill Koch's postulate.

In addition, for those diseases whose pathogens have been fully characterized (e.g., white pox, plague type II) samples involved a small number of affected corals from one or a few locations, and it is not known whether the disease in corals exhibiting the same field characteristics and signs from other locations is caused by an identical pathogen.

The incidence and severity of reef degradation associated with coral disease has increased considerably over the last two decades. Coral biologists today are not adequately prepared to combat the expanding threat nor equipped with the specialized training in pathology, histology, and disease etiologies to handle the crisis. Currently, there is no mechanism to translate advances in biomedical and veterinary sciences, pathology, toxicology, biotechnology, and resource management to the study of coral disease and health and no sustained funding to support coral disease research programs. As a result, the most basic procedures to ensure sample integrity for diagnosis are not followed; basic research tools including a model research organism, cell cultures, and bioindicators for diagnostics, have not been developed; and there is an insufficient capacity of existing coral research laboratories for handling samples and conducting research. We must therefore modify current approaches to studying reef degradation by integrating technologies capable of elucidating underlying mechanisms of disease and couple these efforts to the identification of stressors and determination of causality.



For many diseases, the causative agent(s) remain unknown, such as yellow-blotch disease, while several different microorganisms have been identified as the cause of other diseases, like black-band disease. Adding to the confusion, a coral may be infected by multiple disease at one time: the *Montastraea faveolata* colony to the left was first infected by YBD and subsequently by BBD. *(Photo: Andy Bruckner).*

2. Challenges and Recommendations

The Disease Identification and Disease Investigation Working Group (DIDIWG) acknowledged that the coral reef research community is in critical need of:

(1) a common descriptive language for coral syndromes;
(2) standard disease assessment and reporting protocols; and
(3) designation of core laboratories with critical assay capabilities.

They also recognized the need to constitute a subcommittee to coordinate outreach and education activities (workshops, training curricula, and symposia) in coral health and disease. The ultimate aim of these activities is to provide a consistent, integrated body of scientific information that will guide the development of management strategies for the recovery of the world's coral reef ecosystems.

STRATEGIC OBJECTIVE 1: Standardize common terms and descriptive interpretations of clinical abnormalities noted in field observations.

RECOMMENDATION: Publish a manual with a glossary of common coral health terms and a review of known clinical syndromes.

The lack of standardization of common medical terms and descriptive interpretations of clinical abnormalities noted in field observations has resulted in ambiguous and often misleading communication of findings. To address these deficiencies, the DIDIWG recommended compiling a manual containing both a glossary of common health terms and visual and descriptive details of existing clinical syndromes. The review of clinical syndromes will emphasize data supported by peer-reviewed publications. The manual will promote a standard basic vocabulary and a descriptive understanding of observations used in communicating information relative to coral health and disease.

The DIDIWG recommends that the CDHC form a committee to develop standardized nomenclature in coral health and disease studies. It also recommends that the Nomenclature Committee:

(1) develop a glossary of common medical terms applicable to describing coral condition; and

(2) review existing clinical syndromes.

The Nomenclature Committee should make their report available to the public through the CDHC website: http://www.coral.noaa.gov/coral_disease/cdhc.shtml.

Glossary of Disease Terms

Disease – any deviation from or interruption of the normal structure or function of any body part, organ, or system that is manifested by a characteristic set of signs and whose etiology, pathology, and prognosis may be known or unknown.

Epizootic – attacking many animals in any region at the same time; widely diffused and rapidly spreading. 2. a disease of high morbidity which is only occasionally present in an animal community. (Epidemic is a term reserved for use in reference to people.)

Epizootiology – the scientific study of factors determining the frequency and distribution of diseases among animals. (Epidemiology is a term reserved for use in reference to the study of factors determining the frequency and distribution of diseases in the human community.)

Etiology – the science dealing with causes of disease. The assignment of a cause: as, the etiology of a disease.

Incidence – the number of new cases of a specific disease occurring during a certain time period, or number of diseased individuals in a population.

Koch's Postulates – to establish the specificity of a pathogenic microorganism, it must be present in all cases of the disease, inoculations of its pure cultures must produce disease in animals, and from these it must be again obtained and be propagated in pure culture.

Lesion – any pathological or traumatic discontinuity of tissue or loss of function of a part.

Morbidity – the condition of being diseased; unhealthy or unwholesome, also the rate of sickness.

Morphology – the form and structure of a particular organism, organ or part.

Mortality – the death rate; the ratio of the total number of deaths to the population of a specified area in a given time period.

Necrosis – the morphological changes indicative of cell death.

Pathogen – any disease-producing agent or microorganism. pathogenic, adj.

Pathogenicity – the quality of producing or the ability to produce pathologic changes or disease.

Prevalence – the total number of cases, often expressed as a proportion, of a specific disease in existence in a given population at a certain time.

Sign – an indication of the existence of something; any objective evidence of a disease, i.e., such evidence as is perceptible to the examiner, as opposed to the subjective sensations of the patient (symptom). Sign, not symptom should be used to describe lesions or conditions observed in coral, as the ability to convey sensations (feelings) is not a characteristic of coral.

Syndrome – a set of signs occurring together; the sum of signs of any morbid (disease) state.

A glossary of disease terms is one means to establish a common language among coral disease researchers and promote effective and accurate communication.

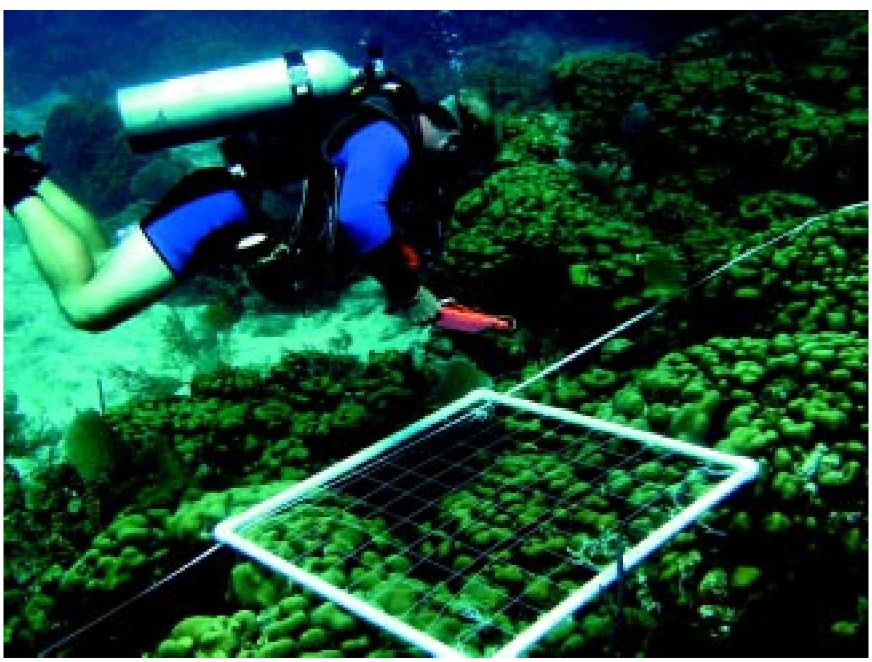
STRATEGIC OBJECTIVE 2: Establish standardized monitoring protocols for field assessments.

RECOMMENDATION: Standardize field collection practices and protocols, and develop a database for storing, compiling and disseminating data.

To understand spatial and temporal patterns of bleaching and disease, it is necessary to make field assessments in a *consistent and comprehensive* manner. Unfortunately, there is a diversity of monitoring protocols being used, by a variety of interest groups, for assessing condition of corals and other reef organisms. This coupled with a lack of common terms to describe conditions affecting organismal health results in data that cannot be compared, making it difficult to determine the impacts of diseases on a region-wide basis. Field assessment personnel must be equipped with a good working -knowledge of the basic terms used to describe coral conditions and be able to apply them accurately during reef monitoring. Clear and consistent descriptions of coral health conditions and syndromes must be developed and implemented, along with standard recording procedures and formats.

The DIDIWG recommends that the CDHC establish a Field Assessment Committee to develop standardized assessment protocols based on a review of existing monitoring programs and integration of established epidemiological principles. In addition, the DIDIWG suggests that this committee will incorporate rigorous Quality Assurance/Quality Control (QA/QC) procedures to maximize data integrity. Implementation of a coordinated and standardized field assessment protocol will ensure that the information being collected can be integrated with other data for bioinformatic analyses.

The DIDIWG recommends that the CDHC establish a Bioinformatics Committee to develop and organize a CDHC database that will interface with existing coral databases and provide synthesis, exploratory, and inferential analysis capabilities.



Scientist at Virgin Islands National Park using meter-square quadrats along permanent transects to monitor the coral disease, White Plague, at Tektite Reef, St.John. This reef, dominated by *Montastraea annularis* illustrates the complexity of disease monitoring techniques based on counting the number of coral colonies *(Photo: Jeff Miller)*.

STRATEGIC OBJECTIVE 3: Establish Regional Coral Disease Diagnostic Centers

RECOMMENDATION: Improve response to disease outbreaks by establishing regional diagnostic laboratories that are staffed with knowledgeable diagnosticians and equipped with modern clinical diagnostics.

In order to achieve the goal of understanding coral diseases and then using this information to develop technologies for curing and/or preventing the spread of these diseases, there must be a strong laboratory component to the CDHC. Establishing regional laboratories would ensure local access to diagnostic services and close cooperation between field and laboratory research groups.

The DIDIWG envisions that these laboratories would function in:

(1) setting diagnostic criteria and standard operating procedures for sample handling, transport, and analysis;
(2) obtaining and archiving voucher samples of healthy and diseased tissue from field surveys and experiments;
(3) performing clinical diagnostic tests on diseased specimens;
(4) assist in developing and testing of new bioindicators for coral disease diagnosis;
(5) performing research investigations on the etiology of coral diseases (emphasizing the most devastating diseases and coral bleaching), remembering that it is important to not only determine the causative agent, but also the mechanisms of the disease, how it is transmitted, and factors that exacerbate the occurrence and impacts; and
(6) providing a learning environment for researchers interested in entering the field of coral disease, in particular microbial ecologists, molecular biologists and infectious disease scientists. Some of the responsibilities of this function include efforts to:

- Develop a draft curriculum for cross-disciplinary training for graduate degrees with a specialty in coral health & disease.
- Organize an annual meeting of coral reef health & disease specialists (consider a Gordon Conference format).
- Identify sources of funding for these programs.
- In all cases, efforts should be made to ensure international collaboration.

Each laboratory is envisioned to implement strict clinical procedures through the use of standardized protocols for the transfer and evaluation of samples from the field to the laboratory.

The DIDIWG recommends that the CDHC form a Laboratory Committee with five sub-committees responsible for developing Standard Operating Procedures (SOPs) for sample collection and transport including a Field Assessment, Histopathology, Microbiology, Molecular, and Toxicology Committee.

STRATEGIC OBJECTIVE 4: Devote training programs to the mechanisms underlying coral disease.

RECOMMENDATION: Educate the general public about infectious versus non-infectious causes of coral diseases and provide cross disciplinary training for scientists and managers charged with caring for coral reefs.

The DIDIWG emphasizes that there is a need for training programs in coral health and disease. There are many programs available on coral reefs, but few are devoted to the mechanisms underlying disease in corals. There is a critical need to provide training for the general public on the nature and impacts of infectious versus non-infectious diseases affecting coral reef ecosystems. Similarly, there is also a critical need to provide cross disciplinary training for scientists conducting coral reef research and monitoring, and managers who are charged with the care of reefs as well as the next generation of scientists.

Dr. Esther Peters provides training in coral histology to students attending a summer workshop at the Hawaii Institute of Marine Biology Coconut Island, Hawaii. *(Photo: Teresa Lewis)*.

The DIDIWG recommends that the CDHC form a Training Committee that will:

- Identify the courses available on coral reef health and disease;
- Recommend a method for coordinating short courses or workshops;
- Develop a website for communicating information about coral health and the activities of the CDHC;
- Develop draft curricula for a training or certification program in coral health & disease; and,
- Develop training materials for courses (videos, identification cards, etc.), compiling existing training information to serve as the foundation.

Disease Identification and Disease Investigation Working Group Members (DIDIWG)

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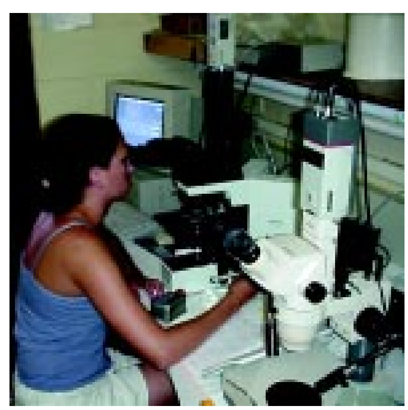
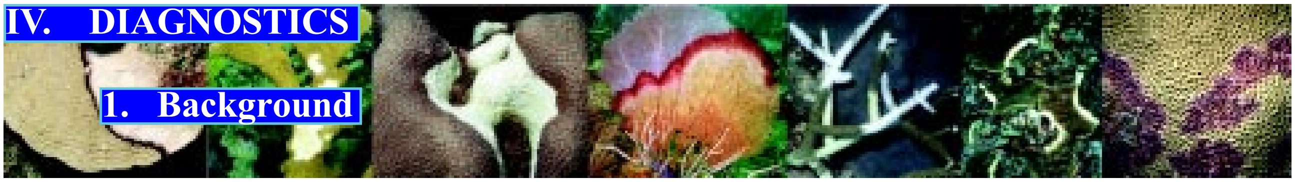
Pam Parnell – Clemson Veterinary Diagnostic Center
Laurie Richardson – Florida International University
Eugene Rosenberg – Tel Aviv University
Emmett Shotts (Chair) – U.S. Geological Survey (Retired)



A student counting extracted zooxanthellae using a compound microscope. *(Photo:Teresa Lewis)*.

IV. DIAGNOSTICS

1. Background



Coral diseases have increased significantly over the last decade and are causing widespread mortality of important reef-building species. The frequency, intensity, and geographic range of coral disease and bleaching events appear to be accelerating at unprecedented rates. The etiologies of most coral diseases including the biotic and abiotic factors contributing to their occurrence and spread have not been fully discerned. Some pathogens of scleractinian corals and gorgonians have been identified; however, the agent(s) of most coral diseases are undescribed. Many abnormalities are not adequately characterized and are indistinguishable to the untrained eye. Because coral health and disease have not been well documented in the past, the diagnostic processes for investigation of disease outbreaks are still in their infancy.

Diagnostics may be defined as: "A variety of observations and tests used to determine the composition and function of an organism and its environment, which allows determination of the organism's health and/or disease state, including susceptibility to disease." Thus, diagnostics are biological tests used to define boundaries of disease, and to define the state of health. It is a **process** by which potential causes are eliminated. The steps involved include:

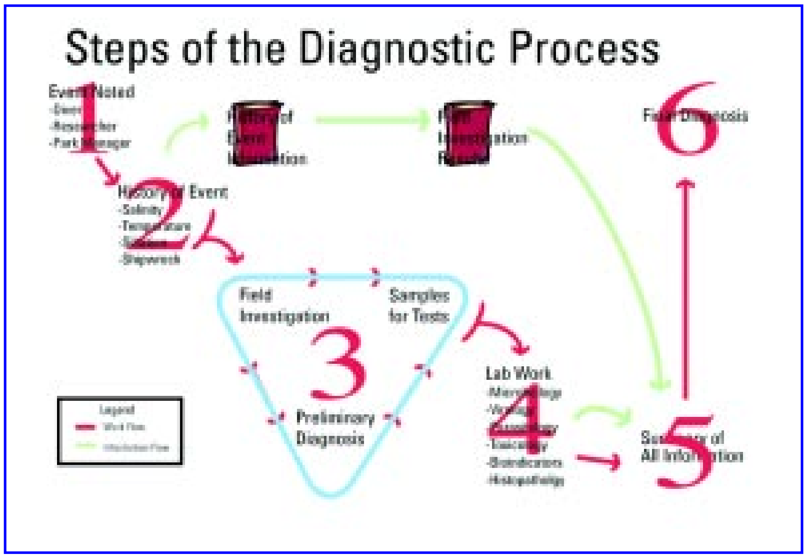
a) collecting comprehensive case histories;
b) recording gross observations;
c) developing a flow chart of steps used to identify patterns;
d) implementing diagnostic tools; and
e) deriving a final diagnosis from total body information.

Two diagnostic strategies are commonly employed.

Community Level Diagnostics: Analysis of community level response to a disease event provides historical information including the identification of stages of declining health. Information is generally gathered after an outbreak or deaths have occurred. To the coral biologist this represents the analysis of the impact of declining health or disease on coral ecology.

Individual Level Diagnostics: Analysis of individual organisms focus on identifying causative agents, characterizing mechanisms of disease, and elucidating patterns of transmission to identify a probable cause and suggest preventative strategies or treatments. This type of diagnosis generally reflects both an analyst's and pathologist's points of view, e.g., defining coral as abnormal through gross observation, collection of samples, processing of tissues, and microscopic observation coupled with clinical tests.

The study of coral diseases requires a modified approach from that taken for terrestrial organisms as well as other aquatic organisms during the initial phases of the disease investigation. The coral diagnostician must have a broad knowledge of the aquatic environment in order to relate clinical findings to disease entities. Initially classifying visual categories and deviations from normal patterns in the field (aquatic environment) is necessary to relate tissue damage to stressors. Therefore, dependence upon correlations between community level diagnostics and individual diagnosis is critical for the marine organism.



Step 1: Sick coral are noticed and reported to the proper authority.

Step 2: The environmental (ecological) historical background of the event is investigated. This type of information is epizootiological. Gathering this information might be done by a marine biologist or a disease specialist. The background information provides the first clues about what is going on.

Step 3: A Coral Health Specialist will examine the coral in situ, study the lesions, take photographs, make a provisional diagnosis and collect the proper samples to confirm that diagnosis. For a coral event, samples are collected for the bioindicator tests that form an important part of a coral health investigation and for microscopic examination.

Step 4: Lab work is conducted by a number of diagnosticians with different expertise. Histopathology may serve in Steps 4, 5 and 6. In many laboratories the pathologist that did the necropsy (sample examination) will also do the microscopic examination of the tissues, review all the available information about the event, and make the final diagnosis.

Step 5: A summation of all available information is used to confirm the preliminary diagnosis. In animal and human diagnostics this is usually done by a pathologist.

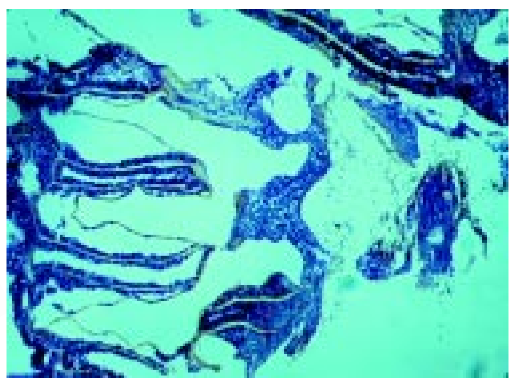
Step 6: Making the final diagnosis.

Step 7: The final diagnosis and as much of the other information as possible should be stored in electronic databases for epizootiological and other kinds of research.

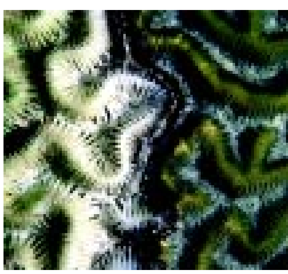
2. Challenges and Recommendations

The *Diagnostic Working Group* (DWG) was charged with identifying gaps in knowledge, research needs, and challenges that impede improved understanding of coral health and the ability to diagnose coral diseases. The lack of standard terminology and diagnostic criteria required to describe coral diseases/syndromes have constrained researchers' ability to delineate specific disease cause and effect. This situation is complicated by the regular reporting of field and laboratory information using non-comparable methods. In addition, associated environmental information related to physicochemical condition of sediments, water column, and nearby land masses is often unavailable.

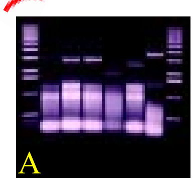
The standard diagnostic processes used in medical and veterinary pathology have not been routinely applied to the study of coral disease. The challenge for the study of coral disease is to develop standard observation, sample collection, and analytical protocols based on a standardized diagnostic approach, yet tailored for corals, and paralleling those used in medical and veterinary pathology. Most of the individual level diagnostic tests cannot be conducted in the field thus samples must be shipped to laboratory sites, often outside of the country of sample origin. Since transportation of coral across international borders is regulated by CITES and collection of coral is regulated by individual state laws, the permitting process may be a significant deterrent to rapid response to disease outbreaks. The established steps in the diagnostic process, identified above, were evaluated by the DWG in terms of coral disease, leading to the following set of Strategic Objectives and relevant recommendations.

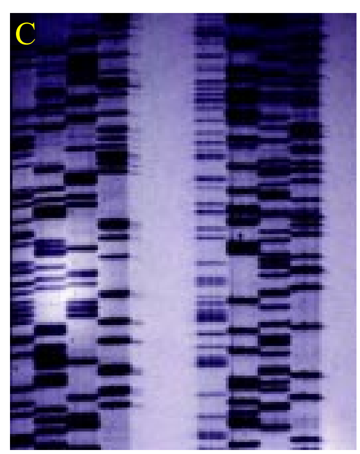


Histological section of black-band disease on *Diploria strigosa*. Lysed tissue caused by the microbial consortium in the mat on the left is pale and has lost structure in comparison to normal tissue on the right. Stained with Taylor's modification of Brown and Brenn's for Gram-positive and Gram-negative bacteria. *(Photomicrograph: Esther Peters).*



Diploria strigosa with black-band disease. *(Photos: Andy Bruckner).*





One way to verify the identification of a pathogen is by sequencing its DNA. In this example, nucleic acids from the cyanobacteria associated with BBD and RBD were isolated and run on minigels to observe purity (a). After extraction of DNA, 16s rRNA was amplified using the polymerase chain reaction. The amplified material was introduced into host *E. coli* cells that are grown on agar (b). The amplified plasmid from the *E. coli* cells was extracted and the 16s rRNA sequence was determined using Sequenase T7 polymerase and denaturing polyacrylamide gel electrophopresis (c). *(Photos: Andy Bruckner).*

STRATEGIC OBJECTIVE 1: Improve methods of taking case histories by developing response teams, implementing standard monitoring protocols, and integrating ecosystem level data.

RECOMMENDATIONS:

a) Develop Standard Operating Procedures (SOPs) for sample collection.

The DWG recommends the establishment of a Field Assessment Committee in support of the CDHC to develop standard protocols and associated data collection forms incorporating rigorous QA/QC procedures for data collection. Determining whether the amount, type, and quality of data are appropriate and sufficient to test the hypotheses under investigation is an important aspect of research. Numerous techniques have been developed for coral collection; however, methods typically are not planned in conjunction with other studies, such as microbial or chemical assays. Many quantitative techniques are used to evaluate the distribution and impacts of disease; however, standard procedures must be developed for assessing water and sediment conditions, recording visual observations (i.e. photography), and taking physicochemical measurements. In addition, standard protocols for sample collection must be implemented to accurately compare aspects of coral health and disease across broad geographic regions.

b) Establish rapid response teams and regional diagnostic centers.

The DWG recommends the establishment of coordinated teams of researchers, resource managers, dive masters, and recreational divers that could be mobilized on short notice to investigate bleaching and disease events. To enhance the timing of the field response, the teams would consist of a core group responsible for coordinating activities as well as several regional teams that are able to respond in their particular areas. Teams would be responsible for rapidly assessing reef condition utilizing SOPs and directing sample collections using appropriate diagnostic approaches. The objective is to integrate field researcher's (e.g., ecologists, marine biologists, chemists, oceanographers) and laboratory researcher's (microbiologists, pathologists, and veterinarians) descriptions of the disease state and conditions associated with the event.

Students at a Molecular Techniques workshop held in Hawaii. Students are learning standardized approaches and techniques for laboratory studies on coral diseases. *(Photo: Teresa Lewis).*

c) Integrate ecosystem level data to assist in diagnosis.

The DWG recommends that the CDHC develop a standardized list of ecosystem level data that should be collected to complement descriptive and quantitative organismal level information collected on coral condition, changes in coral health, and coral mortality. Types of data could include:

1) benthic community composition including various coral measures such as cover, diversity, abundance, size frequency; algal community composition; composition of the coral cover, and abundance of non-coral invertebrates with emphasis on taxa that play a key role in the structure and function of reef ecosystems;

2) fish community dynamics;

3) water and sediment measures, including circulation patterns, wave data, temperature, nutrient analysis;

4) linkages among species, such as the density or abundance of coral predators, key missing trophic levels or organisms (e.g., *Diadema*), and unusual occurrences such as an outbreak of a pest species;

5) socio-economic data, including extent of fishing and gear types; recreational uses; and land-based activities; and human impacts.

Diver recording data at a CARICOMP site in the northern Bahamas *(Photo: Gary Ostrander)*.

Wherever possible, ecosystem level understandings should be coordinated at large spatial scales (regional studies) using a random stratified approach with standardization of methodologies, parameter measures, and quality control measures that would be comparable among related studies.

STRATEGIC OBJECTIVE 2: Implement standard terminology for describing changes in coral health.

RECOMMENDATION: Organize a manual providing common terminology for use in coral field research to promote efficient communication with other researchers, medical/veterinary correspondents, and resource managers.

The DWG recommends that the CDHC form a Nomenclature Committee to develop standardized terminology in coral health and disease studies, publish this information on the CDHC website (http://www.coral.noaa.gov/coral_disease/cdhc.shtml), and in a booklet for distribution. This manual should include descriptions, photographs and diagnostic criteria to assist in field identification of recognized diseases, as well as a separate section discussing other described syndromes and anomalies, signs of predation, overgrowth, competition and other sources of mortality, and newly identified but unpublished syndromes. The booklet could also include a second level of detail involving laboratory diagnosis of peer-reviewed coral diseases. The Nomenclature Committee should also include a glossary of common human medical and veterinary terms applicable to coral diseases. This recommendation coincides with the one made by the DIDIWG.

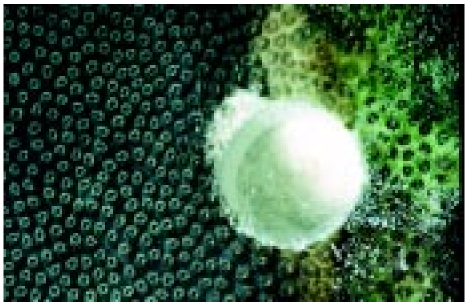
STRATEGIC OBJECTIVE 3: Incorporate standard protocols, routine collection permits, cross-border transportation, and adequate bio-containment measures in coral sampling.

RECOMMENDATIONS:

a) Establish standard coral sample collection protocols using standardized terminology.

The DWG emphasizes the need for a standard coral sample collection protocol to allow analysis and comparison of findings and information sharing among researchers and managers. This should include the development of a sampling program that incorporates the visual observations that should be made for each affected coral (e.g., color of tissue and affected area, shape, distribution, size, and pattern of the lesion); how to photograph the sampled corals (including distance and macro-photographs before and after sample collection); how to remove, collect, preserve and transport coral tissue, skeleton and mucus samples; sediment and/or water samples that should be collected; and techniques to minimize damage to host coral and the associated community.

Diver removing cores from *Montastraea faveolata* using a pnuematic drill. *(Photo: Andy Bruckner).*



Example of core removed from *M. faveolata* at the interface between normal tissue, diseased tissue and exposed skeleton. *(Photo: Andy Bruckner).*

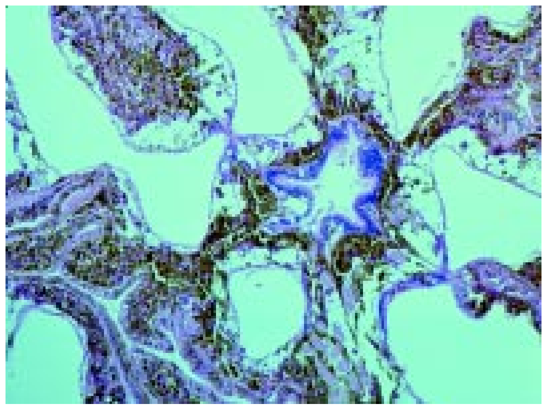
Diver using a leather punch to remove a small sample from a colony of *Montastraea faveolata.* This approach may be preferable to the example shown above, as it is less invasive. *(Photo: Richard Curry).*

Divers involved in a disease investigation collecting samples of staghorn coral (*Acropora cervicornis*) using a pair of clippers and wearing disposable gloves to minimize gear contamination and inadvertant transmission of disease to healthy colonies. *(Photo: Charles Fasano).*

b) Develop an atlas of diseased and normal coral tissue.

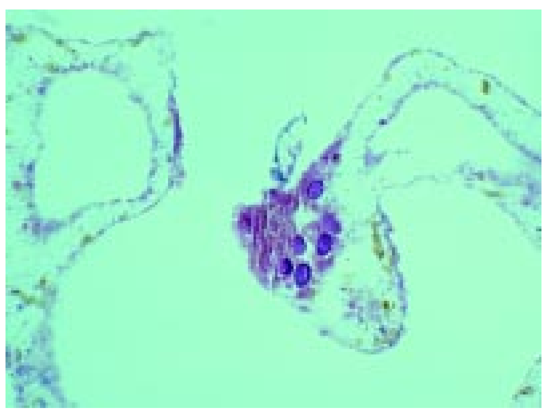
Knowledge of the microscopic structure and composition of coral tissues has been accumulating for more than a century, but many questions remain to be explored. For example, the basic anatomy and histology of corals is fairly straightforward, while the composition and functions of the various cell types and the variation found among different species, genera, and families require further analysis and coordination with studies of biochemistry and metabolism. A comparison of cells and tissues from organisms considered to be healthy with those from organisms whose health is considered to be impaired has been instrumental in the diagnosis and treatment of diseases in other organisms. These same approaches should be applied to corals to study the adverse effects of chemicals or pollutants, to understand the functioning of the immune system in response to pathogens or for other purposes.

Live colony of *Oculina* spp. Puerto Rico, 15 m depth.*(Photo: Andy Bruckner).*



Section through normal pharynx of a polyp on *Porites astreoides* (left). Note brown granular chromophore cells. Stained with Harris's hematoxylin and eosin. (*Photomicrograph: Esther Peters).*

Decalcified *Oculina varicosa*. Decalcification is one of the first steps in preparing coral for histo-pathological examinations. This is a fragment of coral with its skeleton removed, showing the extensive network of tissue buried inside the protective skeleton. *(Photo: Dorothy Howard).*



Gregarine cysts within the calicoblastic epidermis of *Porites porites*. Stained with Heidenhain's aniline blue. (*Photomicrograph: Esther Peters).*

To assist in advancing our understanding and better interpretation of health and disease in corals, the DWG recommends creating a repository of coral tissue samples, and providing a forum for discussion and dissemination of information on coral health and disease. The reference collection could include an archive of histological slides representing healthy and abnormal tissues from representative coral species. This collection would be available on loan for researchers. In addition, a journal dedicated to coral health and disease could be developed with online access provided through the CDHC.

c) Develop non-invasive sampling techniques and educate divers and investigators on bio-containment issues.

Sample collection procedures should incorporate preservation and conservation practices and use appropriate bio-containment and safety measures. One way this can be achieved is by establishing vessels designed specifically for coral research, equipped with appropriate sampling tools and laboratories incorporating various levels of bio-safety practices. However, because the vast majority of coral researchers are not operating out of large research vessels, *standardized protocols to minimize diver impacts and reduce potential for introduction or spread of pathogens must be developed.*

Diver with two small (2.5 cm diameter) cores removed from *Montastraea faveolata*. Mona Island, Puerto Rico. *(Photo: Andy Bruckner).*

This should include:

- environmentally friendly tissue sampling protocols that minimize the size of the core removed and provide techniques for patching the lesions with clay, cement or underwater epoxy;
- methods to prevent dispersal or spread of microorganisms associated with coral mucus or band diseases (e.g, BBD) when collecting samples or treating corals; and
- proper approaches for disposing of used fixative.

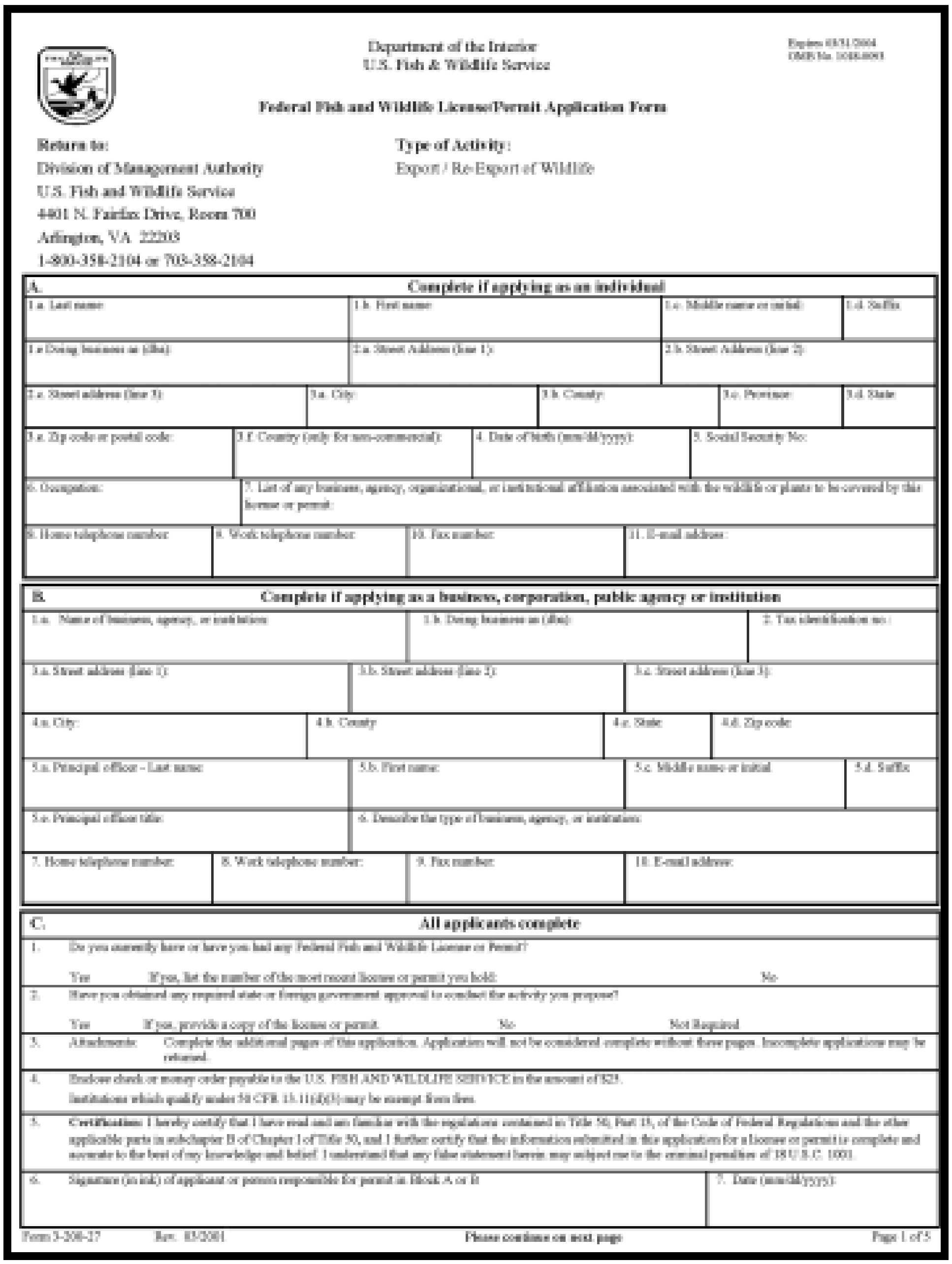
It is also recommended that samples are collected only by researchers with expertise in coral biology, physiology, taxonomy and/or diseases. Volunteer and recreational divers, dive masters, and others conducting monitoring as well as non-coral disease experts on rapid response teams should not collect samples unless they have the proper training and supplies or are accompanied by an expert with proper training.

Diver treating a colony of *Montastraea faveolata* affected by yellow-blotch disease using underwater putty. *(Photo: Andy Bruckner).*

Diver is removing a small tissue sample from a colony of *Montastraea faveolata* using a rock hammer and small leather punch. *(Photo: Richard Curry).*

d) Involve the State Department, U.S. Fish and Wildlife Service, and the CITES Secretariat in facilitating the collection permit process.

All stony corals are listed on Appendix II of the Convention on the International Trade in Endangered Species (CITES) (http://www.cites.org/eng/disc/text.shtml) which requires that researchers transporting corals across national borders acquire an export permit from the country of origin. While the U.S. does not require import permits, other locations such as countries in the European Union do require this. In addition, permits for coral research must also be obtained in writing from the appropriate state, federal or territorial government prior to the start of a research project. While this often places a great burden on researchers, these permits are necessary to avoid prosecution or confiscation of samples, and are required for publication of the data. This process can be facilitated by educating government officials to the present gaps in knowledge and reinforcing the need for action. By bringing together officials and organizations at all levels of the research and management process, effective coral assessment studies can be developed and maintained, and processes for obtaining permits can be facilitated.



Department of the Interior
U.S. Fish & Wildlife Service

Expires 03/31/2004
OMB No. 1018-0093

Federal Fish and Wildlife License/Permit Application Form

Return to:
Division of Management Authority
U.S. Fish and Wildlife Service
4401 N. Fairfax Drive, Room 700
Arlington, VA 22203
1-800-358-2104 or 703-358-2104

Type of Activity:
Export / Re-Export of Wildlife

A. Complete if applying as an individual

1.a. Last name: | 1.b. First name: | 1.c. Middle name or initial: | 1.d. Suffix
1.e Doing business as (dba): | 2.a. Street Address (line 1): | 2.b. Street Address (line 2):
2.c. Street address (line 3): | 3.a. City: | 3.b. County: | 3.c. Province: | 3.d. State
3.e. Zip code or postal code: | 3.f. Country (only for non-commercial): | 4. Date of birth (mm/dd/yyyy): | 5. Social Security No:
6. Occupation: | 7. List of any business, agency, organizational, or institutional affiliation associated with the wildlife or plants to be covered by this license or permit:
8. Home telephone number: | 9. Work telephone number: | 10. Fax number: | 11. E-mail address:

B. Complete if applying as a business, corporation, public agency or institution

1.a. Name of business, agency, or institution: | 1.b. Doing business as (dba): | 2. Tax identification no.:
3.a. Street address (line 1): | 3.b. Street address (line 2): | 3.c. Street address (line 3):
4.a. City: | 4.b. County: | 4.c. State: | 4.d. Zip code:
5.a. Principal officer - Last name: | 5.b. First name: | 5.c. Middle name or initial | 5.d. Suffix
5.e. Principal officer title: | 6. Describe the type of business, agency, or institution:
7. Home telephone number: | 8. Work telephone number: | 9. Fax number: | 10. E-mail address:

C. All applicants complete

1. Do you currently have or have you had any Federal Fish and Wildlife License or Permit?
Yes If yes, list the number of the most recent license or permit you hold: No
2. Have you obtained any required state or foreign government approval to conduct the activity you propose?
Yes If yes, provide a copy of the license or permit. No Not Required
3. Attachments: Complete the additional pages of this application. Application will not be considered complete without these pages. Incomplete applications may be returned.
4. Enclose check or money order payable to the U.S. FISH AND WILDLIFE SERVICE in the amount of $25.
Institutions which qualify under 50 CFR 13.11(d)(3) may be exempt from fees.
5. **Certification:** I hereby certify that I have read and am familiar with the regulations contained in Title 50, Part 13, of the Code of Federal Regulations and the other applicable parts in subchapter B of Chapter I of Title 50, and I further certify that the information submitted in this application for a license or permit is complete and accurate to the best of my knowledge and belief. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001.
6. Signature (in ink) of applicant or person responsible for permit in Block A or B | 7. Date (mm/dd/yyyy):

Form 3-200-27 Rev. 03/2001 **Please continue on next page** Page 1 of 5

Example of a CITES permit from the United States for wild animals required by researchers transporting live corals or coral samples across international borders.

STRATEGIC OBJECTIVE 4: Establish a centralized data system that incorporates a bioinformatic approach that compiles relevant data, transforms the data into information, and formulates a synthesis of the information into a multidimensional understanding of coral health and disease.

The DWG recognizes the importance of interdisciplinary studies, incorporating information from analytical chemistry, biochemistry, microbiology, molecular biology, physiology, and new developments in histopathology techniques, as well as oceanography and ecology, to enhance our understanding of the effects of environmental stressors and disease on coral reefs. The DWG identified the need for the application of new tools to analyze and interpret these data as they relate to the occurrence, spread and impacts of coral diseases. In addition to typical correlative analyses that are frequently performed with ecological data sets, multidisciplinary data should be compiled into relational databases and examined for non-linear correlations using a bioinformatic approach.

Bioinformatics is the study of the inherent structure of biological information and biological systems that brings together systematic biological data with analytical theory and practical tools of mathematics and computer science. The goal of bioinformatics is to apply tools and techniques from computer science, mathematics and statistics to analyze and manage biological data. The development of an 'intelligent' system that can assimilate data into knowledge about coral health and disease involves three phases in a bioinformatics approach:

1) the collection of data of all types, including those not necessarily compatible, into specific databases;

2) the interpretation of specific data sets using typical statistical and comparative approaches; and

3) the drawing of conclusions using many data sets and pieces of information (metadata) to develop new concepts.

The recommendations for this objective have been formulated around these three phases.

RECOMMENDATIONS:

a) Develop standard methods for data collection that will enable centralization of data, via several centralized repositories; compile protocols from published field and laboratory methods, focusing on procedures related to coral assessment.

Standard methods and protocols should be developed for all aspects of clinical diagnostics including histopathology, microbiology, molecular biotechnology, and toxicology, as well as field diagnostics. The DWG recommends that the CDHC establish committees to develop Standard Operating Procedures (SOPs) and establish a centralized means of data collection that integrates clinical data with biological, genetic, environmental and climatic data in formats conducive to bioinformatics and exploratory analyses.

b) Assimilate and analyze data, compare with other relevant parameters, and interpret data and publish the results in standard formats.

There are a variety of databases and websites that provide general information on coral reefs, as well as several targeted programs such as the United Nations Environmental Programs World Conservation Monitoring Centre's (WCMC) Global Coral Disease Database (http://www.wcmc.org.uk/marine/coraldis/), The World Resources Institute's Reefs at Risk program (http://www.wri.org/wri/reefsatrisk/), Reefbase (http://www.reefbase.org/), and other programs that have detailed information on specific aspects of corals, coral reefs, and natural and anthropogenic stressors affecting these ecosystems. One of the problems often encountered when compiling or analyzing these types of information is the lack of standardization in data collection, making interpretation difficult. Another limitation is the recognition that environmental and anthropogenic stressors are prevalent on most coral reefs to varying degrees, yet relationships with coral disease and health are incompletely known.

The DWG recommends that all available information on coral reefs and specifically on coral physiology, disease and health, and environmental and anthropogenic stresses operating on these systems be assessed using a bioinformatic similative approach. By providing computational algorithms, databases, data mining, statistical and visualization tools, bioinformatics can be used to answer questions on coral disease and health such as relationships among environmental and anthropogenic stressors and gene expression. Furthermore, once a database and necessary analysis and visualization tools are developed, new information can be added as it becomes available.

STRATEGIC OBJECTIVE 5: Draw upon data, information, and knowledge generated by coral studies conducted under uniform standards and reported with standardized terminology to develop 'coral specific' diagnostics.

RECOMMENDATIONS:

a) Conduct special emphasis workshops with the goal of applying medical/ veterinary diagnostic processes to coral disease study/investigation.

The DWG recommends that the CDHC convene a meeting of disease experts, including coral physiologists, coral disease researchers, medical doctors, veterinarians, and other researchers with medical and immunological backgrounds to develop diagnostic criteria and procedures that would be applicable to the study of coral diseases. At minimum, this workshop would bring together existing approaches and develop specific guidelines and protocols for application of histopathology, microbiology, molecular biotechnology, and toxicology techniques to the understanding of coral disease processes. For example, the group could recommend the use of a particular diagnostic biomarkers to diagnose the state of health or identify disease (e.g., heat shock, carcinogen metabolism, general health status); validate specific biomarkers with disease identification; or evaluate new biomarkers that indicate disease susceptibility or can be used in the diagnostic process.

b) Establish a working committee that will maintain a list of recognized coral diseases/syndromes, screen new additions to the list, and report regularly to the CDHC.

The DWG recommends the creation of a Nomenclature Committee that will review and evaluate all of the described coral diseases/syndromes and develop a standardized, agreed upon list of names. This list will include as a first tier the peer-reviewed coral diseases for which a causative agent has been identified and additional diseases/syndromes that have been described in peer-reviewed literature, but for which a confirmed pathogen has not yet been identified. As a second tier, other diseases/ syndromes that have been reported by monitoring programs, researchers, and other divers, but have not appeared in the peer-reviewed literature will be described. The standardized coral disease terminology that is developed will include reference to synonyms of syndromes that have been used in the past but are being replaced by standardized nomenclature, as well as a description of other coral anomalies, signs of predation, competitive organisms and other causes of mortality. The list will be based on standard terminology incorporating veterinary and medical classifications as appropriate.

This committee will also be responsible for reviewing new additions and providing regular updates to the CDHC. The established list of coral diseases/syndromes and other conditions will be maintained on the CDHC website, along with photographs and descriptions to assist in field identification, illustrations of pathogens (if available), gross and microscopic characteristics, including histological changes to coral tissue in response to diseases, toxins, and other stresses, and other diagnostic information when possible.

Diagnostics Working Group Members (DWG)
Kay Briggs – US Minerals Management Service
Colleen Charles – US Geological Survey
Phil Dustan – College of Charleston
Judy Halas – Environmental Moorings International
Laura Kracker – NOAA/National Ocean Service
Kristy Lide – Medical University of South Carolina
Lynda Lanning – Otsuka Maryland Research Institute
Shawn McLaughlin (Chair) – NOAA/National Ocean Service
Esther Peters – Tetra Tech Inc
Charles Robinson – EnVirtue Biotechnologies, Inc
Lou Sileo – US Geological Survey
Garriet Smith – University of South Carolina, Aiken
Darren Wray – NOAA/National Ocean Service

White-band disease, Mona Island, Puerto Rico. WBD is believed to be one of the most significant causes of decline in *Acropora palmata* and *A. cervicornis*, but researchers have been unable to identify a causative agent. *(Photo: Andy Bruckner).*

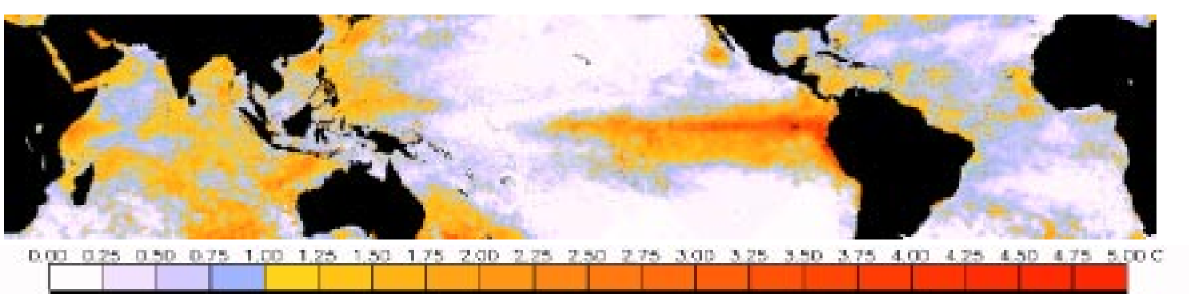
V. ENVIRONMENTAL FACTORS AFFECTING INFECTIVITY AND SUSCEPTIBILITY

1. Background

Over the last 220,000 years, geological records show that the Caribbean coral community structure has been stable, despite vast changes in natural environmental conditions. However, in the last 30 years, coral communities have been dramatically altered. The most disconcerting observations suggest that corals are not recovering. Disturbances of various types, including hurricanes, coral bleaching, diseases of corals and other organisms, and other natural stressors have been invoked to explain the decline in coral cover and concurrent increases in fleshy and filamentous macro-algae (Aronson and Precht, 2001). However, the substantial coral decline and reef changes observed since the 1970s point convincingly to human activities as one of the major causes. A growing number of threats to coral reefs have been documented and evaluated over the past decade, including both anthropogenic and climatic changes. These threats translate into multiple stressors with synergistic impacts, and include physical factors (i.e., elevated temperatures, sedimentation, UV, overfishing, storm damage, vessel groundings), chemical factors (i.e., pesticides, herbicides, nutrients, pharmaceuticals in sewage, oil spills, and industrial discharge) and biological factors (i.e., disease, bleaching, predation, algal and invertebrate competition). In addition, effects of anthropogenic impacts are often exacerbated by climate change events (i.e., El Niño Southern Oscillations) which are increasing in intensity and frequency. This, coupled with the fact that corals live in low nutrient, relatively stable and uniform conditions, makes them vulnerable and very sensitive to physical and/or chemical changes in their environment.

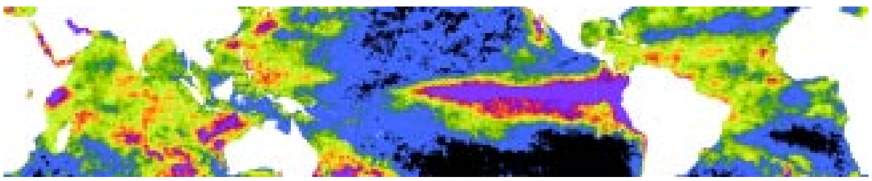
Climate change is suggested to be the cause of many problems affecting coral reefs today (Reaser et al., 2000). Global climate change has led to increases in sea surface temperature (SST), sea level, atmospheric CO_2, solar irradiance, and other environmental features of critical importance to coral health. Short-term effects of these environmental changes can alter basal metabolic function, including effects on coral respiration and algal symbiont photosynthesis. Longer-term stress can increase disease susceptibility and mortality rates or reduce growth and reproductive rates.

The earth's atmosphere and oceans have warmed significantly in the last century; the estimated average increase in ocean temperature was 0.5° C, with more pronounced increases in tropical and subtropical water, where most coral reefs occur (Pittock, 1999). The most dramatic change in climate on record occurred when a major El Niño event, starting in early 1997 and lasting 12 months, was followed by a La Niña event in 1998 that lasted 11 months. During these climatic events there was a major coral bleaching event associated with extensive tissue mortality of corals, mostly in the Indo-Pacific. Coral bleaching events of varied scope and



1998 Satellite Annual Composite HotSpot Chart (derived from Pathfinder AVHRR SSTs). *(Photo: Gang Lui and Al Strong).*

scale were also documented in early 2000, 2001, and again in early 2002, with the most dramatic effects in the Indian Ocean and the Western Pacific (Hoegh-Guldberg, 1999). Coral bleaching and mortality is, however, only one of many global effects of elevated sea surface temperature. According to *Status of Coral Reefs of the World: 2000*, the most alarming report is that increasing concentrations of CO_2 in seawater have reduced rates of coral calcification (Wilkinson, 2000). This condition slows growth, reduces skeletal density, leading to increased coral fragility and decreased reef-building capacity. Atmospheric CO_2 concentrations have risen exponentially in the last century and a half. This has caused a decrease in ocean pH and total alkalinity. Simulations indicate these elevations of ocean geochemistry will significantly reduce coral calcification, independent of temperature effects (Gattuso et al., 1999).



1998 Satellite Annual Composite Degree Heating Week Chart (DHW)derived from Pathfinder AVHRR SSTs. *(Photo: Gang Lui and Al Strong).*

Although it is clear that diseases are emerging at an accelerated rate, cause and effect relationships are not well documented, especially in terms of what effect environmental changes have on the occurrence, proliferation and impact of coral diseases. It is now recognized that coral disease has played an important role in the loss of coral cover, especially in the wider Caribbean. For instance, mortality from white-band disease (WBD) has modified the structure and composition of reefs throughout the Caribbean by contributing to the loss of two of the most common and abundant species, *Acropora palmata* and *Acropora cervicornis*. Yet factors that triggered or exacerbated the impacts of WBD are unknown, and coral populations were affected by WBD on reefs near human population centers as well as offshore reefs in protected watersheds. The role of environmental stressors must be carefully evaluated before concrete conclusions of relationships with disease can be made. Most coastal environments are affected by a suite of anthropogenic inputs, making it difficult to identify any one specific cause associated with deteriorating health or an increased prevalence of disease. Differences in prevalence, severity, or impact of coral diseases may be related to specific anthropogenic stressors, other biotic or abiotic parameters, or more likely, multiple factors.

Throughout the Caribbean high relief *Acropora cervicornis* dominated habitats (left) have been transformed into rubble fields by disease and other factors and recovery has failed to occur (above). *(Photos: Andy Bruckner).*

2. Challenges and Recommendations

A persistent lack of knowledge about the history of coral health and diseases has prevented scientists from understanding the changes to reefs being seen today. In addition, our ability to understand these changes is stymied by the lack of historical data to compare these gross changes. Because there are no standard records of disease or changes in the growth or distribution of corals in many regions, few attempts have been made to correlate coral susceptibility with known environmental changes.

While examining environmental factors affecting coral disease and health, the Environmental Factors Working Group (EFWG) identified the lack of urgency to protect and conserve coral reefs as the most serious impediment facing coral reefs worldwide. They suggest that those with the means to achieve protection and conservation of coral reefs are implementing a reactive approach instead of proactive measures to insure coral reef survival for future generations. The EFWG agreed that the most important challenge was to convince policy makers of the real and urgent threat of losing the world's coral reefs caused by declining health of corals.

OVERALL RECOMMENDATION: Develop An Approach To Understand Relationships Between Environmental Factors And Coral Disease And Health.

OVERALL STRATEGIC OBJECTIVE: Provide compelling evidence to convince the public, policy makers, managers, and regulators that declining coral health and increases in disease are caused primarily by changes in land-use patterns that contribute to degraded water quality through eutrophication, sedimentation, and chemical loading.

In the following sections, the EFWG provides recommended mechanisms to achieve the Overall Strategic Objective. This includes eleven key strategies that are critical to advancing our understanding of environmental factors that affect coral health by (1) increased susceptibility to disease, (2) greater impacts from coral diseases, and (3) critical to developing and implementing strategies to mitigate these impacts. The eleven strategic objectives are subdivided into field and laboratory categories.

FIELD

STRATEGIC OBJECTIVE 1: Conduct epizootiological studies to determine the relationships between coral disease, coral health and changing environmental conditions.

RECOMMENDATION: Support more comprehensive epizootiological studies relating coral health and coral diseases to environmental conditions, especially associations involving changes in water quality and climatic parameters.

There have been very few epizootiological studies examining the relationships between coral health and environmental parameters. A recent study that mapped the spatial distribution of coral diseases in the western Atlantic, using published reports, found that 97% of all locations affected by disease correspond to areas where human activities are expected to have medium to high impacts, based on the *Reefs at Risk* analysis (Green and Bruckner, 2000). However, a causal link cannot be drawn from this observation, as it is at least partially due to the fact that most coral disease research has been conducted on reefs located adjacent to developed coastlines, and fewer studies have been carried out in remote locations.

Because of the paucity of recent epizootiological studies, and a lack of historical data, it is unclear what "background levels" of disease prevalence are normal for reefs and the threshold levels that indicate an epizootic. In addition, scientists currently cannot discriminate among disease events that are natural and those indicative of anthropogenic stress causing elevated responses, except in the case of certain mass mortalities. Furthermore, most studies from the 1970s-1990s reported a fairly low prevalence of disease (often less than 1%) with localized outbreaks affecting up to 50% or more of the corals, but these studies rarely included information on environmental parameters that may have been associated with the outbreak.

Environmental stressors, including those associated with degraded water quality and/or climatic parameters, are most often cited as potential factors causing coral mortality and death. Unfortunately, there are not adequate measurements and studies correlating coral health and disease to exposures to environmental stressors. Even when disease epizootics have been observed, few environmental measurements have been made, and even fewer are made on a regular basis to distinguish an irregular trend over time.

During periods of heavy rainfall, sediment, nutrients, pesticides, sewage and other land-based pollutants may be transported from land to coral reef habitats, affecting water quality (as depicted in the photograph to the left) and possibly contributing to increased bleaching, diseases and coral mortality. *(Photo: Andy Bruckner).*

A better understanding is required of the impacts of environmental stressors. There is a need for additional field studies that examine the prevalence and incidence of coral disease and changes in coral health, and simultaneously collect data on different environmental parameters to understand cause and effect relationships. There is also a need for studies that evaluate the effect of chronic stressors (i.e., sedimentation and nitrification) on coral health, including their impact on coral survival and recovery following major natural disturbances such as hurricanes or predator outbreaks.

Diver collecting sediment traps to determine sediment loading. *(Photo: Kathy Price).*

STRATEGIC OBJECTIVE 2: Determine whether recent declines in coral health are unprecedented, or whether similar declines occurred in the past in the absence of anthropogenic stressors.

RECOMMENDATION: Consult literature, historical records, cultural knowledge and sclero-chronology for evidence of past mortality events; changes in growth, reproduction, and distributions of corals; and presence and prevalence of disease. Correlate this information with known historical environmental changes.

The EFWG concluded that there is insufficient evidence to support the fact that the destruction of thousands of hectares of modern coral reefs, documented over the last several decades is unprecedented in geological history. While location-specific cores (i.e. Belize) have revealed no evidence of population declines over the last 2500 years; in some locations (USVI and Florida) there are gaps in the fossil record. For instance, coring off Buck Island, USVI has shown that some shallow reef communities were initially dominated by *Acropora palmata* over the last 7000 years, but its presence was interrupted, with it disappearing from the reef system 3000 years before present and then reestablishing after nearly a 1000 year hiatus (D. Hubbard, unpubl. data).

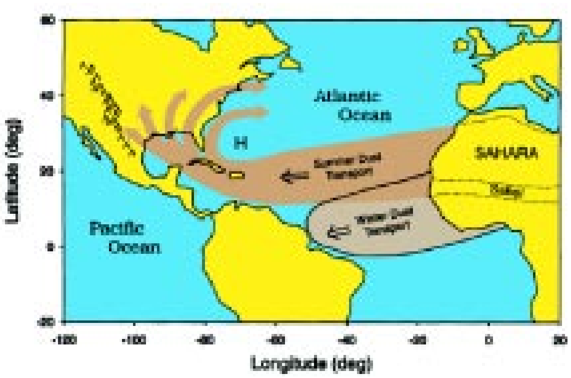
Through the development of a larger, regional scale coring program, it would be possible to compile a long term record and compare this to present day changes, by correlating environmental change with fossil records. While certain environmental variables (i.e. temperature, changes in sea level and antecedent topography) are preserved in the geochemistry of fossils, additional research is needed to determine whether evidence of coral diseases are preserved in the geochemistry or in the composition or nature of fossil assemblages. This information could provide evidence as to whether observable disease signs are new or if they have historical precedent, and also may help determine background levels of past disease prevalence. Literature dating back to the early 1920's discussed the presence of bacterial aggregates in corals, and used the term "bacteriosis." Molecular approaches to evolutionary studies of corals and known pathogens, such as quantifying levels of "biomarkers" that remain stable over time, can be used to understand changes seen today.

Coral geologists removing a core from a large *Montastraea faveolata* colony. *(Photo: Andy Bruckner).*

STRATEGIC OBJECTIVE 3: Support research to identify possible vectors for disease-causing pathogens, with an emphasis on understanding the role of environmental factors in the spread and virulence of these pathogens.

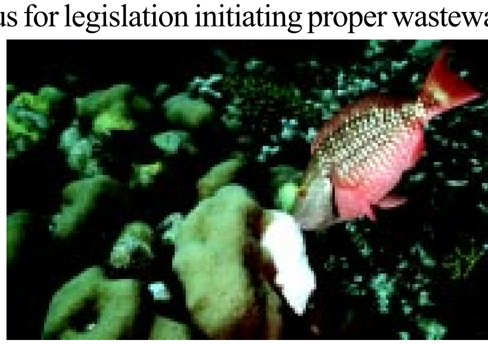
RECOMMENDATION: Support a multidisciplinary effort to conduct controlled experiments that evaluate possible sources of pathogens and relationships among environmental factors and the dispersal and spread of pathogens, including terrestrial runoff and sewage discharge, aerosols and dust, ballast water, invasive species, and biotic vectors.

Research should be targeted toward understanding patterns of susceptibility to disease and factors that promote the proliferation and spread of pathogens, examining possible disease vectors, natural reservoirs and possible external sources for the introduction of disease-causing agents. Studies should include an examination of the potential introduction via aerosols and dust, terrestrial runoff (including point and non-point source pollution), ballast water, or pathogens transported through introduction of exotic species or through aquaculture and mariculture. Emphasis should also be placed on understanding the role of other reef organisms, such as parrotfish, damselfish and snails, as a source of and vector for the dispersal of pathogenic microorganisms. In recent literature, at least two known pathogens are reputed to have been transported into the reef environment from external sources. This includes *Aspergillus* spp., a fungus that affects sea fans and other gorgonians which may have been introduced via terrigenous runoff or through deposition of African dust. Also, *Serratia marcescens*, the causative agent of white pox in Florida, is a common intestinal bacteria that inhabits the human gut and is also found in reef fishes and other organisms (Patterson et al., 2002). Additionally, it has been reported that 60% or more of all marine phytoplankton die from viruses, and that zooplankton consume both phytoplankton and bacteria. Zooplankton themselves are a primary food source for those coral species most affected by coral diseases. Pelagic dispersal of such contaminated food fits with limited epizootiological data and disease models, and yet remains largely unexplored.



Schematic diagram of the transport of African dust to the Caribbean (modified from Perry et al). Some researchers suggest a link between disease outbreaks and African dust, and at least one putative coral pathogen (*Aspergillus*) is known to occur in dust.

Through the identification of possible sources and vectors of pathogenic organisms, managers can begin to develop strategies to prevent their transmission. For instance, if the causative agent of white pox is found to be human in origin and is introduced into the marine environment via sewage discharge, this could become the impetus for legislation initiating proper wastewater treatment systems in the Florida Keys.



Many fish and invertebrates feed on or around corals, such as the stoplight parrotfish (*Sparisoma viride*) which is known to feed on live coral (right) and algae. Through routine feeding, the behavior of parrotfish may serve as a vector for disease-causing pathogens. *(Photo: Andy Bruckner).*

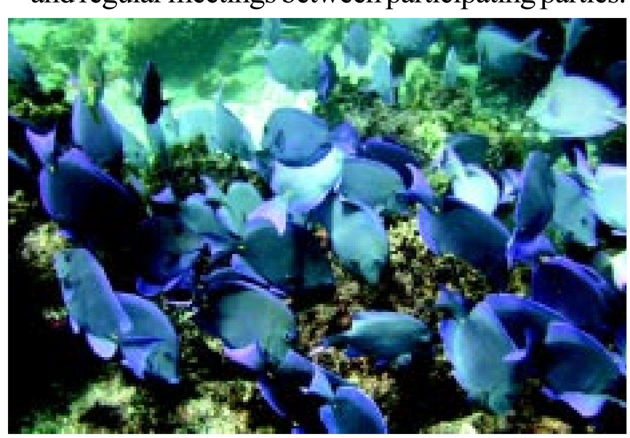
STRATEGIC OBJECTIVE 4: Support international efforts to allow ecosystem level understandings that ignore political boundaries.

RECOMMENDATION: Support research, monitoring, and assessment efforts aimed at understanding large-scale ecosystem phenomena, and foster strategic partnerships with other countries, international organizations and institutions to conduct large scale studies and to share expertise and information.

The EFWG emphasized the importance of removing jurisdictional limits on the scientific investigation of ecosystem processes, since ecosystems know no political boundaries. Coral reefs are closely linked to water circulation patterns which influence larval transport and retention, and the dispersal and spread of putative pathogens and pollutants. Although it is possible to identify direct causes of mortality (e.g., bleaching, diseases, hurricane damage), reefs are often highly variable in their responses to apparently similar stressors. Our limited understanding of ecological processes and interactions at the population and community level, and oceanographic processes that affect ecological connectivity of reefs, reduces our ability to identify and understand unique indicators of coral health and mortality. Furthermore, most coral disease studies in the western Atlantic to date have focused on Florida, which may not be representative of the rest of the Caribbean, much less the vast Indo-Pacific region, emphasizing the need for more international efforts.

Mangroves (above) are a key component of coral reef ecosystems. They provide critical nursery areas for commercially important species and also trap sediment and pollutants from land, among other values. *(Photo: Andy Bruckner).*

Since coral reefs extend beyond state and national boundaries, the strongest efforts should be coordinated at adequate spatial scales (i.e. regional studies) to integrate surveys, assessments, and monitoring of environmental parameters and their impacts on coral health and disease. Those efforts should be coordinated with mechanistic studies of disease dynamics and they need to insure that standardization of methods, parameters measured, and quality control measures are consistent and comparable between the different studies. Adequate funding should be included for training and regular meetings between participating parties.



Blue tangs feeding on algae (right). Coral disease studies should integrate ecosystem-scale data including information on fish communities, algae, and benthic invertebrates. *(Photo: Andy Bruckner).*

STRATEGIC OBJECTIVE 5: Establish a standard for scientific information, communication and field observations to provide efficient and effective communication among scientists, managers, dive operators, and recreational divers; to coordinate information; and to maximize resources for understanding relationships between coral health and environmental influences.

RECOMMENDATION: Develop and maintain a specific *Coral Disease and Health Consortium* (CHDC) website and list-server for information exchange and to facilitate communication about coral health between coral reef workers.

The creation of a CDHC website would facilitate communication between workers in different disciplines actively researching issues of coral health and disease. This group could include resource managers, researchers, dive masters and other professionals. Many resource managers and dive shop operators are the eyes and the ears of the coral reefs. They visit the reefs on a regular basis and can offer accounts of unusual sightings or report changes from normal conditions. Often, the lack of communication and technical training to adequately report detailed observations of coral reef condition to the scientific community prevents the information and sample flow to those who are trained to study the problem. Greater benefits to respond and learn about these events could be obtained by educating resource managers to adequately report to and collect samples for the scientists who study coral diseases. The resource managers then could train appropriate individuals to precisely describe conditions and abnormalities of coral health, to document these events, and obtain samples for histopathological, microbiological, molecular, and environmental condition studies.

The proposed website* also could be used to link or advertise the presence of different databases available to use in epizootiological studies. For example, databases containing information regarding water quality, climatic change measurements, other environmental parameters, or information about coral health that are maintained by different groups could be disseminated for applications in other studies.

WCMC maintains the Global Coral Disease Database. This site contains records of all published accounts of coral disease, as well as unpublished monitoring data and it allows users to map the distribution of coral diseases. This site could be linked to other GIS databases with environmental data and other information.

* The Draft CDHC website can be accessed at: http://www.coral.noaa.gov/coral_disease/cdhc.shtml.

STRATEGIC OBJECTIVE 6: Standardize monitoring, assessment/collection protocols, reporting standards, and nomenclature of disease/syndromes including disease signs and diagnosis.

RECOMMENDATION: Form a committee to study and recommend standardized monitoring, assessment and collection protocols for epizootiological studies, and to standardize coral disease nomenclature.

There is a need to establish a committee to improve the collection and reporting of data on coral health and disease. This committee should address the types of environmental parameters that should be monitored, how to collect data, and how to analyze the biotic and abiotic data collected. The committee should also prescribe specific procedures to ensure data consistency and quality. Any program to monitor environmental parameters should use consistent techniques with sufficiently high resolution to detect significant changes. The data obtained should be amenable to statistical analyses and they should be accessible at a future date to allow a comparison of existing conditions with conditions at that time. Standardizing these approaches would allow the integration and comparisons of numerous studies in order to understand large-scale epizootiological perspectives. The committee would recommend standardized data collection protocols for physical, chemical and biological parameters; analytical chemistry protocols to ensure the precision, accuracy, sensitivity, and quality of comparable measurements; and other guidance to ensure high data quality are comparable across different regions and laboratories. It is proposed that a hierarchical approach, based on several criteria, should be developed to direct different levels of efforts. The committee's recommendations should consider factors such as cost effectiveness, ease of use, and facilities and equipment required to define a prioritized and tiered approach to set the parameters of importance to monitor. It is recommended that the committee use information already available or that is being developed by national or state monitoring programs that have an obligation to assess the coastal conditions of their marine resources.

To promote meaningful descriptive observations used in the scientific literature, the EFWG recommends the implementation of standardized disease nomenclature that follows established aquatic health and/or veterinarian standards. Often the incorrect use of terms or description of conditions due to inadequate definition in the literature prevents a clear understanding of the abnormality being described. In many cases, what is presumed to be the same condition has been reported by different names, depending on the observer, making it difficult to integrate data. It is recommended that the list server/website be used to coordinate these efforts between scientists. Furthermore, because of recent controversy surrounding several proposed or described syndromes, it is imperative that the terminology is developed in coordination with all participants of the CDHC.

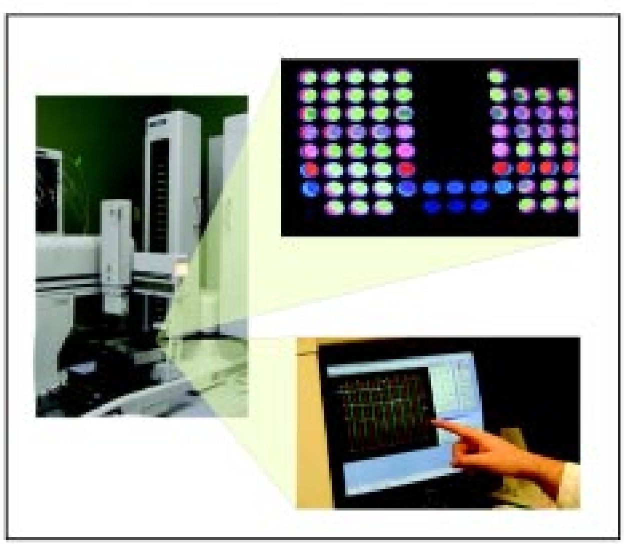
A researcher setting up a gel to assess the purity of DNA extracted from a pathogenic organism. (*Photo: Teresa Lewis)*.

STRATEGIC OBJECTIVE 7: Obtain adequate funding to ensure that cost-effective technologies are available to assess and understand relationships between coral health and environmental parameters (e.g. water quality and climate).

RECOMMENDATION: Increased funding to support technological development for assessment of biological, chemical, and physical resources applicable to the study of coral reef environments.

A priority should be placed on funding the development and improvement of instrumentation, biosensors, and other innovative technology to increase sensitivity and/or reduce cost for *in situ* assessments of coral reef health and disease. Monitoring and diagnostic technologies applicable to study coral reefs are non-existent. The unparalleled environmental conditions and unique biodiversity of coral reefs creates many challenges that must be mastered to overcome the existing technological limitations. For example, most instrumentation used to measure *in situ* water quality does not have the sensitivity to measure the low levels of analytes present in oligotrophic reef environments. Additionally, high salinity and rapid fouling of instruments typical in these environments complicate the deployment and maintenance of equipment for any length of time. Examples of technologies under development are: *in situ* probes designed to measure a suite of nutrients on regular intervals; antifouling devices for dissolved oxygen meters; and aerial surveillance devices needed to assess coral condition. These and other technologies, e.g. biosensors for detecting changes in coral health status, should be added to the resource manager's "tool box" to enable them to monitor environmental conditions in real time.

NOAA's Coral Reef Early Warning System (CREWS) station in St. Croix. CREWS stations collect hourly meteorological and oceanographic data (air temperature, wind speed, wind direction, barometric pressure, sea temperature, light [above and below the water], salinity, pulse-amplitude-modulated [PAM] fluorometry, and CO_2 partial pressure [pCO_2]). What makes these CREWS stations unique is that expert system software screens the incoming data to not only look for anomalous data, but also for matching patterns (which serve as models and environmental indices) to discern what environmental combinations result in biological phenomena observed at the CREWS station site, for instance coral bleaching. *(Photo: Jim Hendee and Monika Gurnee)*.



Sensitive ELISA assays are being used by high through-put robotic screening facility to enhance determination of environmental and disease impacts on susceptible coral species. *(Photos: Sara Polson).*

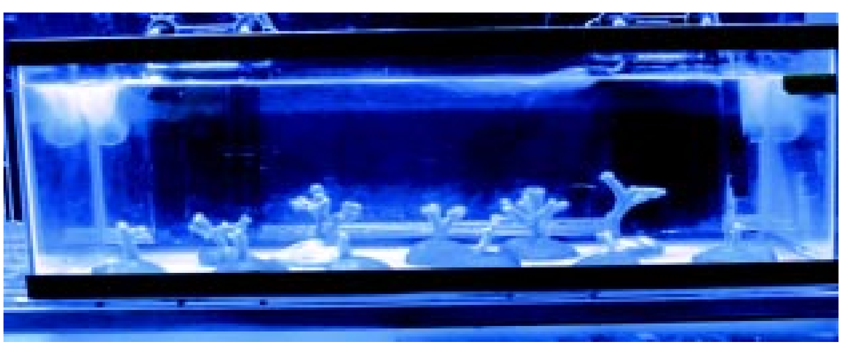
Laboratory Research and Development

Information derived from environmental field studies will stimulate hypotheses formulation required to drive laboratory testing. These studies will enable researchers to prioritize relevant environmental stressors for laboratory assessment.

STRATEGIC OBJECTIVE 8: Establish appropriate model system(s) for standardized coral studies in the laboratory.

RECOMMENDATION: Create model systems for studies relating to the health of reef-building corals and their symbiotic algae. Establish "lab rat" equivalents to be used for standardized, repeatable experiments studying pathogenic coral diseases and their relationship to environmental factors. A facility should be established and supported to supply cultured coral animals. Other model systems including symbiotic algal cultures, coral cell lines, and pathogenic microorganisms should be deposited in existing facilities designed to supply and maintain such biological materials for the research community.

Culture facilities should be established to create, maintain, and supply lines of coral animals that can be used for coral studies by the general research community. The facility should ensure the genetic integrity, health, fitness, and availability of the corals supplied for different studies. The facility should be responsible for assuring the quality of the test animals so that studies made by any lab in the world will be comparable. In addition, established symbiotic algal cultures, coral cell lines, and pathogenic bacterial cultures should be deposited in the appropriate commercial type collections after they are thoroughly described and documented in the literature by their originators. All cultured materials should be made readily available to interested researchers to promote rapid development in the state of knowledge of coral health and disease.

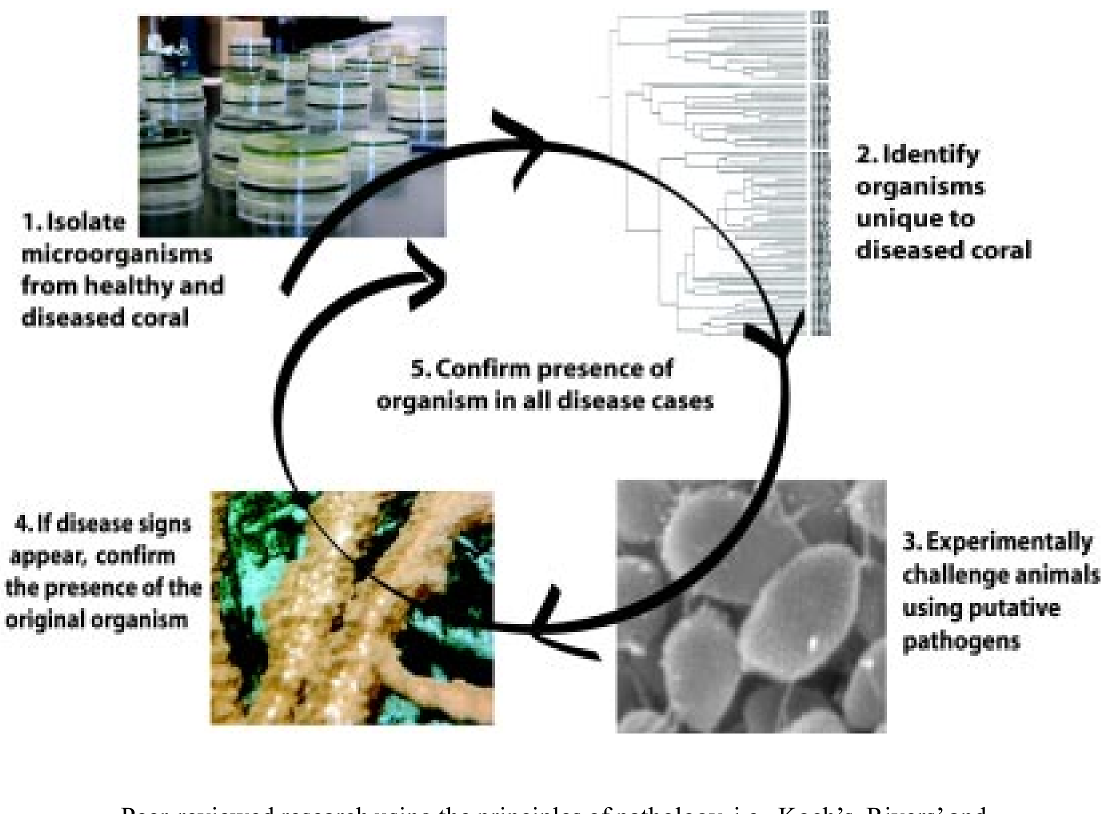


These model systems are important to study the impact of individual and/or multiple environmental stressors on coral health, and how they can exacerbate coral disease. Propagation of test corals in culture facilities will decrease the field collection of this rare and protected resource by overcoming the problems with acquiring enough tissue samples for analyte analyses. The culture facility also should lead the effort in training the scientific community for maintaining coral culture and promoting responsible coral husbandry by setting standards and guidelines, and by conducting training workshops.

STRATEGIC OBJECTIVE 9: Implement focused research to determine coral disease etiologies including infectious and non-infectious diseases.

RECOMMENDATION: Provide competitive peer-reviewed research funding with priorities focused on determining etiologies of infectious and non-infectious diseases of corals, including an emphasis on evaluating mechanisms of resistance and physiological endpoints that are indicative of changes of coral health.

Coral infectious disease studies should follow the same processes and precepts established in human and veterinary medical studies employing Koch's, Rivers', and Evan's Postulates as models for etiological description. A limited number of such studies have been conducted to date. A lack of funding directed toward the study of coral disease has slowed progress in this arena. Several coral disease syndromes may be caused by abiotic or non-infectious factors such as the exposure of coral reef organisms to harmful substances and conditions that occur regularly in our coastal areas. It is recognized that these studies should be undertaken in closed systems to contain hazardous materials and prevent potentially dangerous exposures to the natural reef environment. Little knowledge is available on the genetics of corals or of the impact of changing environmental parameters as an influence on gene expression. Research should be encouraged in disciplines related to health and fitness with the goal of providing needed insight into mechanisms of resistance, immunity, and a detailed understanding of the coral-algal symbiotic complex.

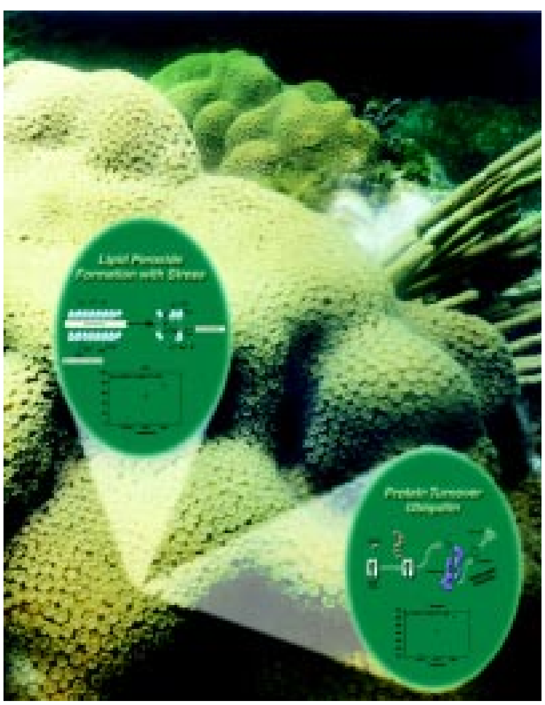


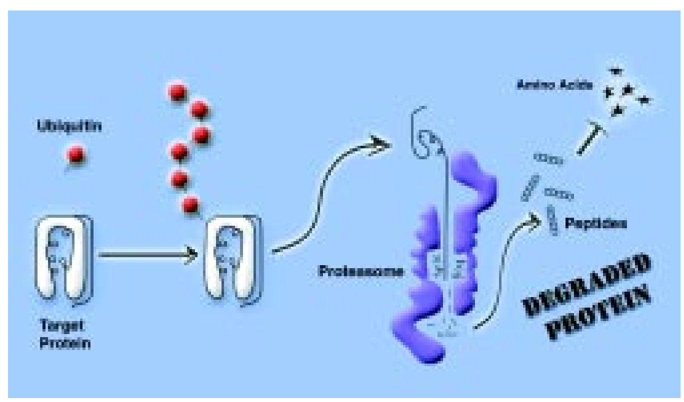
Peer-reviewed research using the principles of pathology, i.e., Koch's, Rivers' and Evan's Postulates, are vital for verifying agents of disease. *(Artwork: Sara Polson)*.

STRATEGIC OBJECTIVE 10: Determine the cause and effects of environmental and climatic stressors on the health of coral and the role of abiotic diseases.

RECOMMENDATION: Provide competitive peer-reviewed research funding with priorities focused on determining the effects of environmental and climatic stressors on coral health using laboratory studies.

In addition to the lack of knowledge concerning coral disease etiologies, even less is understood about the acute and chronic effect of different environmental and climatic stressors. Since many of the real declines in coral coverage and health have been attributed to anthropogenic impacts changing the natural state of the environment or climate around reefs, it is necessary to test hypotheses generated from field epizootiological studies in laboratory settings. Coral model systems should be used to test the effects of these stresses, which range from chemical to physical to biological factors. Acute and chronic exposure studies under controlled laboratory conditions should be used to investigate changes in coral fitness. The advantage to these studies is that corals may be exposed to both individual stressors and complex mixtures to determine health impacts that simulate realistic environmental conditions. These research efforts need to develop and incorporate appropriate end points to identify stress, e.g., biomarkers that correlate health effects with environmental parameters, and biomarkers that signal a decline in the health status of corals. The goal of these studies is to determine how abiotic-induced diseases might be manifested in the field and to provide information about disease susceptibility and persistence when corals are under stressful conditions. Information gleaned during the recovery of diseased corals could lead to mitigative activities.



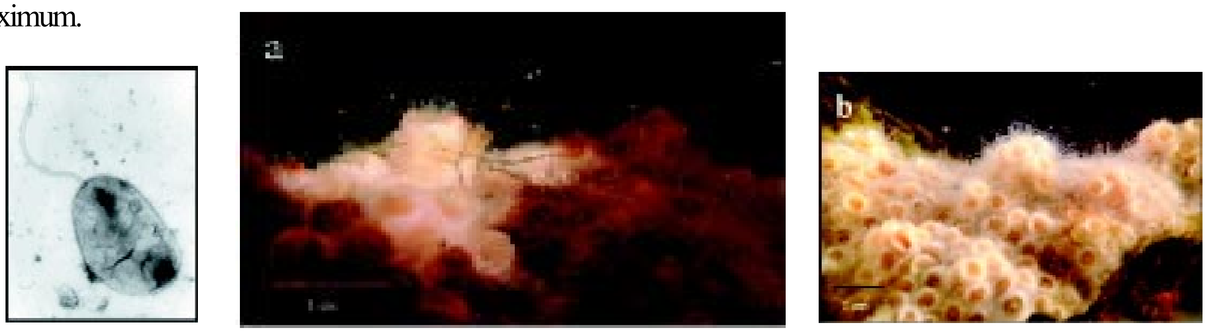


Cartoon depicting the ubiquiting pathway that is involved in protein turnover at a cellular physiological level (top right). It is used when there is a lot of growth or a lot of damage. Samples taken from *Montastraea annularis* show increases of ubiquitin under stressful experimental conditions. *(Artwork: Cheryl Woodley).*

STRATEGIC OBJECTIVE 11: Determine environmental parameters that are optimal for coral growth as well as optimal conditions for known and putative pathogens.

RECOMMENDATION: Support research to understand the relationships between environmental factors and optimal coral health, as well as the effects of environmental factors on pathogenic microorganisms.

Little is known about basic coral biology and physiology, including immunity and symbiotic relationships, or the effects of various environmental parameters on coral growth, reproduction and other functions. The EFWG encourages the study of the basic biology/physiology of corals, including which environmental conditions promote optimal coral growth and those which favor declines in coral health. Research targeted towards understanding the optimal conditions for coral growth, reproduction and other organism-level processes may improve our capacity to predict factors that increase disease susceptibility, as well as possible mechanisms of resistance to infection and/or resilience of corals exposed to disease agents. In addition, the relationships between environmental factors and the proliferation of pathogenic microorganisms should be evaluated to determine optimal conditions for the survival, virulence, and spread of pathogens. While recent studies on black-band disease have identified possible natural reservoirs for the cyanobacteria involved in the disease, as well as the presence of the cyanobacteria on a coral host in a non-infectious state, environmental factors that may trigger a change from a non-infectious to an infectious stage are unknown (Richardson, 1997). The cause of *Vibrio shiloi* infection of *Oculina pategonica* illustrates how environmental conditions, specifically climatic change are linked to virulence and pathogenicity of a normally non-virulent bacteria. This bacterium expresses virulence genes that cause coral disease when SSTs are only slightly elevated above the average SST maximum.



Bacterial bleaching in *Oculina* caused by *Vibrio shiloi*. Light microscope of the causative agent, *V. shiloi* (left), and examples of partially (a) and fully (b) bleached colonies. *(Photos: Eugene Rosenberg)*.

Environmental Factors Working Group Members (EFWG)
Eric Borneman – University of Houston
Andy Bruckner – NOAA/National Marine Fisheries Service
Richard Curry – Biscayne National Park
Craig Downs – EnVirtue Biotechnologies, Inc.
Chris Johnston – Medical University of South Carolina
Frank Morado – NOAA/National Marine Fisheries Service
Pam Morris – Medical University of South Carolina
Debbie Santavy – US Environmental Protection Agency
Gloria Seaborn – NOAA/National Ocean Service
Ernesto Weil – University of Puerto Rico

VI. SUMMARY

The preceding text lists numerous impediments to progress in the area of coral disease and health and addresses each with a series of recommendations. The length of these lists defines the major challenge before us if the goal of the Coral Disease and Health National Research Plan is to be realized. Some might conclude from the many impediments identified that past research has made little progress; however, this is certainly not the case. The scientific and policy disciplines involved are healthy and maturing, though significantly under-funded relative to the expanding problem.

The major recommended strategic objectives can be summarized as follows:

Establishment of standard terminology, methodology and protocols:
There is limited application of human and veterinary medical knowledge to the study of coral health and disease; standard medical terminology has not been used in descriptive interpretations of clinical anomalies noted during field collections or in the laboratory, resulting in ambiguous and often misleading communication of findings. This is compounded by inadequate diagnostic tools and a deficiency in consistent, comprehensive monitoring, assessment and collection protocols. A review of the existing and emerging coral disease syndromes that institutes clinical diagnostic criteria, including visual and descriptive details is paramount to understanding and combating coral disease.

Expansion of knowledge in basic coral physiology, biology and disease etiology:
There is limited information on the physiological parameters that define healthy coral and even less on coral disease dynamics. By expanding our understanding in areas of cellular physiology, genomics and proteomics we will be able to (1) define baseline measures of coral health and vitality such as non-stress levels and deviations that indicate an altered state; (2) identify normal changes in physiological parameters occurring seasonally, annually, and along geographic, bathymetric, and other physical and/or environmental gradients to increase diagnostic accuracy; (3) identify mechanisms of resistance and susceptibility to disease and factors that modulate defense mechanisms; and (4) characterize processes at individual, population and community levels that affect ecological connectivity of reefs and discover critical control points for management strategies.

Development of model coral species:
All aspects of coral disease and health research have been hampered by the lack of an established coral laboratory model species (analogous to "lab rats"), coral cell lines and standardized sources of algal and bacterial cultures that are routinely available for research. There is a critical need to develop models and provide the infrastructure to maintain these living stock collections. Providing well-characterized and documented experimental organisms to domestic and international researchers will enable rapid advances through the use of modern biomedical techniques to investigate coral health and disease and by focusing research on fundamental biological concepts broadly applicable across the taxon.

Development of a Centralized Data/Knowledge System, Website, Repository and Core Diagnostic Facilities:
Understanding mechanisms of coral disease in order to devise appropriate treatment, mitigation or management regimes has been limited by static databases, comprised of independent datasets that are incompatible with modern integrated analyses. New analytical technologies of bioinformatics now allow the development of an "intelligent" system that can assimilate data of all types into information that can be synthesized into knowledge. Application of bioinformatics to the study and investigation of coral health and disease is critically needed to disentangle the multi-factorial nature of coral disease dynamics. The logistics of this approach demands a centralized system that receives data from field units and is supported by regional response teams, diagnostic laboratories with critical assay capabilities and an underpinning of training and voucher specimen archive.

The rate and extent of progress in coral disease research will depend in part on how effectively the recommendations in this National Research Plan are implemented. Our hope is that numerous state and federal agencies as well as private institutes will use this document to identify topics that relate to their particular responsibilities or purviews, and that scientists and private industry will use these ideas to guide their activities as well. No single agency can address all of the identified impediments, though most can be covered by the combined efforts of several organizations. Overlap and omissions in critical scientific information needs and technology developments for treatment, mitigation or restoration of coral reefs are likely unless further coordination is attempted at the agency level. The Consortium has been established to make this possible by providing the focal point around which to organize and coordinate scientific resources nationally and internationally to address coral health issues. Concerted efforts will be necessary to keep the lines of communication and coordination open.

Students preparing presentations on the coral research projects they carried out during the Molecular Techniques Wokshop held in Hawaii *(Photo: Teresa Lewis).*

VII. ACKNOWLEDGEMENTS

We would like to acknowledge the CDHC Workshop Organizing Committee composed of Dr. Kay Briggs (DOI/MMS), Dr. Andy Bruckner (NOAA/NMFS), Dr. Colleen Charles (DOI/USGS), Dr. Sylvia Galloway (NOAA/NOS), Dr. Lynda Lanning (Otsuka Maryland Research Institute), Dr. Shawn McLaughlin (NOSS/NOS), Dr. Deborah Santavy (USEPA), Dr. William Walker (DOI/USGS), Dr. Thierry Work (DOI/USGS), and Dr. Cheryl Woodley, Chair (NOAA/NOS) for their collective efforts in the weeks prior to the workshop. This team set the vision for the CDHC Workshop, developed the agenda and identified experts to invite that could represent key disciplines in the deliberations.

The foundational background information for the participants was provided in the form of six white papers and ten oral presentations.

Dr. Ernesto Weil provided a white paper entitled: *Coral Disease Epizootiology: Status and Research Needs* this paper and Dr. Weil's presentation on *Coral Disease: A Global Perspective* set the stage for the extent to which coral disease is a global crisis.

Dr. Laurie Richardson provided a white paper entitled: *Infectious diseases of reef corals* as well as a talk entitled: *Coral Disease: What We Know* that clarified to the participants the current state of knowledge for known coral disease agents, as well as providing the need for standardized nomenclature and rigorous criteria for disease identifications.

Dr. Eugene Rosenberg provided a white paper entitled: *The Oculina patagonica-Vibrio shiloi model system for coral bleaching*. He also provided a presentation entitled: *Vibrio shiloi: Case Study* both of which documented the thorough investigative process used to identify and document a causative agent for the pathology of coral bleaching.

Dr. Pamela Parnell, a State Veterinary Diagnostician, provided a roadmap to investigate disease outbreaks. Her white paper was entitled: *Pathogens, Predators and Predicaments: The Process of Disease Investigation* and her very stimulating oral presentation: *Disease Investigation: The Process.*

Dr. Esther Peters, a coral histopathologist, provided the white paper: *Coral Disease Diagnostics: Histopathology* and an oral presentation entitled the same where she discussed the state of the technology for corals and pointed out some unique procedures and criteria for scleractinian corals.

Dr. Andrew Bruckner provided an examination of a manager's perspective of coral disease in his white paper: *Priorities for Effective Management of Coral Disease* and a clear presentation for the workshop in his oral presentation, *How is Coral Disease Managed and What are the Management Issues?*

The white papers were supplemented with three additional oral presentations.

Mr. Craig Downs presented, *Abiotic Factors Affecting Susceptibility & Infectivity*, in which laboratory studies of the effect of iron on *Vibrio shiloi* infectivity was used to demonstrate how a limiting nutrient can affect infectivity without increased temperatures.

Dr. Garriet Smith presented a talk on *Disease Identification: Technologies,* in which he provided the most current microbiological and molecular techniques being employed to study coral disease.

Dr. Jonas Almeida presented a stimulating talk entitled: *Bioinformatics & Coral Disease*, in which he showed the power and possibilities of applying bioinformatic approaches to the study of coral disease.

We would also like to thank Judy and John Halas who provided a special presentation at our reception entitled: *Social & Economic Consequences of Coral Degradation.*

We are especially grateful to the Working Group Chairs, the Facilitators and their Rapporteurs that not only presided over the deliberations during the workshop but also provided the group's draft reports that were used for preparing this document. The Biology Working Group was chaired by Dr. John Fauth, facilitator, Dr. Sylvia Galloway and rapporteur, Ms. Maggie Holbrook. The Disease Identification Disease Investigation Working Group was chaired by Dr. Emmett Shotts, facilitator, Dr. Brian Keller and rapporteur, Mr. Diego Gil-Agudelo. The Diagnostics Working Group was chaired by Dr. Shawn McLaughlin, facilitator Dr. Colleen Charles and rapporteur, Ms. Kristy Lidie. The Environmental Factors Working Group was chaired by Mr. Craig Downs, facilitator Dr. Deborah Santavy and rapporteur, Mr. Chris Johnston. A special thanks goes to Kristy Lide for her technical writing and assistance in pulling together the first draft of this document.

We would also like to express our sincere thanks to those individuals working behind the scenes to make this workshop a success. Darren Wray was responsible for all of the audiovisual equipment, set-up and logistics both at the workshop and at the reception as well as providing photography during the entire workshop. We would like to thank Kellee James for her administrative assistance and Samantha Ryan for logistical assistance.

Particular thanks go to Lynda Lanning, Kristy Lidie, and Kay Briggs who arrived two days in advance of the workshop (a holiday weekend) to help with last minute details involving phoning and faxing news releases to the media, xeroxing late arriving white papers and assembling the notebooks for each participant into the wee hours of the morning to have them ready by registration at 8:00 am.

Financial support for this workshop was provided by NOAA through the Coral Conservation Program. We would also like to thank the Living Oceans Foundation and Dr. Mohammed Faisal, for contributing the time and skills of Ms. Kim Hopkins, their graphic artist, to design the logo for the Workshop and for providing folders for the Workshop documents.

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IX. WORKSHOP AGENDA

Tuesday, Jan. 22, 2002

7:30	*Breakfast*
8:00	*Registration*
8:45	**Welcome** Cheryl Woodley / Geoff Scott
9:00	**Workshop Goals & Objectives** Cheryl Woodley
9:30	**Coral Disease: A Global Perspective** Ernesto Weil
10:00	*Break*
10:20	*Participant Introductions*
10:30	**Coral Disease: What We Know** Laurie Richardson
11:00	***Vibrio shiloi*: Case Study** Eugene Rosenberg
11:30	**Abiotic Factors Affecting Susceptibility & Infectivity** Craig Downs
12:00	*Lunch*
1:00	*Participant Introductions (cont.)*
1:15	**Disease Investigation: The Process** Pamela Parnell
2:00	**Coral Disease Diagnostics: Histopathology** Esther Peters
2:30	**Disease Identification: Technologies** Garriet Smith
3:00	*Break*
3:20	*Participant Introductions (cont.)*
3:30	**Bioinformatics & Coral Disease** Jonas Almeida

4:00	**How is Coral Disease Managed? & What are the Management Issues?** Andy Bruckner
4:30	**Mission Review & Workgroup Objectives** Debbie Santavy
6:30	*Transportation to S.C. Aquarium*
7:00	*Reception at the S.C. Aquarium*
7:30	**Social & Economic Consequences of Coral Degradation** Judy & John Halas
9:00	*Return to Charleston Place Hotel*

Wednesday, Jan. 23, 2002

7:30	*Breakfast*
8:00	**Break into Small Group Discussion** Cheryl Woodley

Group and Room Assignments Rooms:

Biology	2-K
Disease I/I	Edmunds
Diagnostics	Fenwick
Environmental Factors	Hampton

Thursday, Jan. 24, 2002

7:30	*Breakfast*
8:00	**Break into Small Group Discussion** Cheryl Woodley

Group and Room Assignments Rooms:

Biology	2-K
Disease I/I	Edmunds
Diagnostics	Fenwick
Environmental Factors	Hampton

Friday, Jan. 25, 2002

7:30	*Breakfast*
8:00	**Reconvene into Large Group & Present your Groups Ideas and Solutions**
10:00	*Break*
12:00	**Say Goodbye & Have a Safe Journey Home**

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APPENDIX I: CDHC Mission, Objectives, Partners and Structure

Proposed U.S. Coral Reef Task Force *Coral Disease and Health Consortium (CDHC)**

1. ***Mission Statement:*** Create a Coral Disease and Health Consortium
- Promote health and sustainable use of Nation's Coral Reef Ecosystems
- Focus on understanding & assessing health of reef-building corals communities & ecosystems
- Understand disease processes including etiology and transmission, synergistic effects of diseases with environmental stresses, and how they impact health of coral reef ecosystems
- Coordinate an interagency research strategy to study coral diseases, coral bleaching
- Biomarkers of coral health

2. ***Background***:
- Executive Order (#13089) decreed to preserve and protect the biodiversity, health, heritage, and social and economic value of U.S. coral reef ecosystems
- Recommendation by Ecosystem Science and Conservation Working Group, in "Research and Monitoring: Proposed Actions" was to form a Coral Disease Consortium
- Increased mortality as a result of coral diseases and coral bleaching
- Over the last decade, coral diseases increased in frequency, distribution, & numbers of coral species affected
- Trend especially evident in Western Atlantic and Caribbean regions
- Emergence of new disease syndromes with new types of pathologies
- Most diseases appearing on coral reefs have not been thoroughly characterized and their etiologies, including the causative agent, often remain unknown.
- International Society of Reef Studies (ISRS) has produced two issue statements detailing the scientific community's concerns of irreversible damage to coral reefs by coral diseases and coral bleaching.

3. Objectives:
The Coral Disease and Health Consortium will provide a comprehensive approach to understanding coral health. The CDHC will focus on disease and bleaching dynamics, the effect of anthropogenic, climatic, and natural factors on the transmission of coral diseases, and the proliferation of the biotic and abiotic agents responsible for the diseases. The approach will use investigations of the disease etiologies of both host and symbiont, disease agents, host and symbiont responses, defense mechanisms, disease agent and host/symbiont interactions, disease transmission, coupled with an understanding of the factors that influence the susceptibility of the host to the disease and the role of anthropogenic factors in the distribution and abundance of diseases.

The Coral Disease and Health Consortium will address coral health issues, with emphasis on the diagnosis and etiology of coral diseases and bleaching. The Consortium would draw on the expertise of scientists from around the U.S. and internationally who are actively involved in bleaching and disease research; provide a focal point for researching and tracking the progression of bleaching and disease events; and help identify environmental influences that affect these phenomena. The Consortium would be developed through an inter-agency planning process.

* presented to the US CRTF at the March, 2000 meeting in Washington D.C.

4. *Structure and Function:* The proposed investigations will:

1) Increase our fundamental understanding of the nature and diversity of coral diseases;
2) Increase our understanding of the disease process (i.e. host susceptibility, role of genetic variants/mutants and virulence factors);
3) Allow for the development of rapid diagnostics, including the development of molecular probes and other means that could be used to identify and verify a specific disease condition in the field;
4) Allow for the development of "curative" treatments and remediative management for infected areas;
5) Increase our understanding of the impact of coral diseases on the coral reef community structure;
6) Describe, on temporal and geographical scales, the type and density of the various forms of coral diseases;
7) Characterize infectious and noninfectious disease agents this will include histopathology, genetics, *in vitro* culturing;
8) Establish a functional genomics/proteomics approach to understanding coral defense strategies and factors affecting defense mechanisms; and
9) Develop a data repository for coral diseases.

5. Who are partners in this effort? The Coral Disease and Bleaching Center will be a cooperative effort involving NOAA (NOS, NESDIS, OAR and NMFS), EPA, the Department of Interior, States and Territories with coral reefs, and other U.S. Coral Reef Task Force Agencies, in addition to partnering with University participants. It is recommended that a Scientific Advisory Board provide oversight, coordination and direction to the Consortium.

6. Structure and Functional Units

Clinical Pathology Unit

- Diagnostic Services: Histopathology, Clinical assays, Collection/Preservation and Specimen Submission Protocol
- Registry of Coral Disease: Repository of voucher or type specimens (tissues and slide collections); provide "type" slides to researchers; develop electronic atlas of coral diseases
- Electronic Map-based database for disease reports/tracking of occurrence, abundance & distribution to evaluate occurrence of epizootics

Risk Assessment Unit

- Compile field data on occurrence; disease surveys on regional & global scale
- Compile environmental data i.e., climatic, land-use patterns, hazardous events data
- Epidemiologically evaluate field, clinical and environmental data to establish cause and effect models of disease outbreaks and transmission patterns.
- Provide an information dissemination mechanism for the public, managers and researchers

Health Assessment Unit (R& D)

- Disease Etiology: Disease Agent Characterization; Transmission Dynamics
- Indicators of Health Status: Host Susceptibility/System Tolerance; Stress, Defense and Detoxification Responses/Tolerances
- Indicators of Coral Health: Biomarkers (Physiology, Genetics)

APPENDIX II: Time Lines for Objectives

OBJECTIVE	Time Line*
Biology Working Group	
1. Determine the mechanism(s) of coral bleaching	1-3 years
2. Develop model laboratory species to spur advances in coral research	1-3 years
3. Establish coral and zooxanthellae cell cultures for use in laboratory research	3-5 years
4. Determine physiological parameters typical of normal, healthy coral	5-7 years**
5. Determine basic host defense mechanisms and immune responses of corals	5-7 years**
6. Determine patterns of coral gene expression, and genetic mechanisms of resistance and susceptibility to disease	8+ years**
Disease Identification and Disease Investigation Working Group	
1. Standardize terms and descriptive interpretations of clinical abnomalities noted field observations	1-2 years
2. Establish standardized monitoring protocols for field assessments	1-3 years
3. Establish Regional Coral Disease Diagnostic Center	1-2 years
4. Devote training programs to the mechanisms underlying coral disease	2 years
Diagnostics Working Group	
1. Improve methods of taking case histories by developing response teams	2-3 years
implementing standard monitoring protocols	1 year
and integrating ecosystem level data	3-5 years**
2. Implement standard terminology for describing changes in coral health	1-2 years
3. Incorporate standard protocols, routine collection permits, cross-border transportation, and adequate bio-containment measures in coral sampling	
a) sample collection protocol	1 year
b) atlas of diseased and normal tissue	3-5 years
c) non-invasive sampling techniques and biocontainment measures	1-3 years
4. Establish a centralized data system that incorporates a bioinformatic approach that compiles relevant data, transforms the data into information formulates a synthesis of the information into a multidimensional understanding of coral health and disease	3-5 years
5. Draw upon data, information, and knowledge generated by coral studies conducted under uniform standards and reported with standardized terminology to develop 'coral specific' diagnostics	5 years

Environmental Factors Working Group

1. Conduct epizootiological studies to determine the relationships between coral disease, coral health and changing environmental conditions .... 3-5 years**
2. Determine whether recent declines in coral health are unprecedented, or whether similar declines occurred in the past in the absence of anthropogenic stressors.. 2-3 years
3. Support research to identify possible vectors for disease-causing pathogens, with an emphasis on understanding the role of environmental factors in the spread and virulence of these pathogens........ 5-7 years**
4. Support international efforts to allow ecosystem level understandings that ignore political boundaries... 1-2 years
5. Establish a standard for scientific information, communication and field observations to provide efficient and effective communication among scientists, managers, dive operators, and recreational divers; to coordinate information; and to maximize resources for understanding relationships between coral health and environmental influences.. 2-3 years
6. Standardize monitoring, assessment/collection protocols, reporting standards, and nomenclature of disease/syndromes including disease signs and diagnosis... 2-3 years
7. Obtain adequate funding to ensure that cost-effective technologies are available to assess and understand relationships between coral health and environmental parameters........ .... 5-10 years**
8. Establish appropriate model system(s) for standardized coral studies in the laboratory.................. 1-3 years
9. Implement focused research to determine coral disease etiologies including infectious and non-infectious diseases.. 2-5 years**
10. Determine the cause and effects of environmental and climatic stressors on the health of coral and the role of abiotic diseases.. 5-10 years**
11. Determine environmental parameters that are optimal for coral growth as well as optimal conditions for known and putative pathogens.. 3-5 years**

*The estimated time required to complete each objectives is dependent on adequate funding to carry out the activity .

** Many of the objectives require long term monitoring and research and multi-disciplinary teams to be fully completed, however parts of these (e.g., etiology of a specific disease; relationships between a specific diseases and certain environmental pollutants; or the development of a new tool) can be accomplished over the short term (1-3 years), given adequate funding levels .

APPENDIX III: Recommended CDHC Committees and Participants

Each Committee was asked to select a chair and to develop a statement of their goals and objectives or responsibilities. The committees were formed and asked to initiate activity with the goal of quickly making information available via the CDHC webpage. Their activities and products will be described in the CDHC Annual Report.

i. Nomenclature Committee

Responsibilities:

- Develop a manual containing a glossary of common medical terms for use in the field of coral health;
- Review the existing clinical syndromes in coral disease including visual and descriptive detail;
- Provide the Bioinformatics Committee with key terms for data collection; and
- Provide web-based access to this information.

Suggested Participants:

Laurie Richardson (Chair), Pam Parnell, Lynda Lanning, Esther Peters, John Halas, Jim Hendee.

ii. Field Assessment Committee

Responsibilities:

- To develop standard assessment protocols and associated data collection forms;
- Incorporate rigorous QA/QC procedures in protocols;
- Develop standard recording procedures and formats;
- Develop protocols for the transfer of samples; and
- Provide bioinformatics team with parameters (i.e. skeleton, tissue, substrate, etc.) that would require data collection in the field.

Suggested Participants:

Erich Muller (Chair), Janet Foley, Andrew Bruckner, Cindy Hunter, Ernesto Weil, James Porter.

iii. Model Systems Committee

Responsibilities:

- Determine whichspecies are most suitable for use as a coral 'lab rat'; and
- Develop a standard operating protocol for the maintenance, acclimation and experimental exposure conditions of model systems, primarily (but not limited to) those for use in laboratory work investigating aspects of coral disease.

Suggested Participants:

Eric Borneman (Chair), Richard Curry, Erich Mueller, Phil Dustan, Alan Pinder, Melody Moore, Frank Morado , Gary Ostrander, Tom Capo.

iv. Education and Outreach Committee

Responsibilities:

- Identify the courses available on coral reef health and disease;
- Recommend a method for coordinating short courses or workshops;
- Develop a website for communicating information about coral health and activities of the consortium;
- Develop draft curricula for a training or certification program in coral health & disease;
- Develop training materials for courses (videos, identification cards, etc.);
- Develop a draft curriculum for cross-disciplinary training for graduate degrees with a specialty in coral health & disease;
- Organize an annual meeting of coral health and disease specialists (consider a Gordon Conference format).
- Identify sources of funding for these programs; and
- In all cases, efforts should be made to ensure international collaboration.

Suggested Participants:
Jo Ann Leong (Chair), Garriet Smith, Ernesto Weil.

v. Histopathology Committee

Responsibilities:

- Develop Standard Operating Procedures (SOP) which provide general method guidance;
- Identify essential procedures for the collection, transport, processing, and analysis of coral samples;
- Provide the Bioinformatics Committee with relevant datafields associated with microscopic analysis.

Suggested Participants:
Lou Sileo (Chair), Lynda Lanning, Pam Parnell, Esther Peters, Taylor Reynolds, Frank Morado, Shawn McLaughlin, Thierry Work.

vi. Microbiology Committee

Responsibilities:

- Develop Standard Operating Procedures (SOP) for microbial sample collection from the field, including:
  - the defining of equipment and supplies needed;
  - the protocols for collection of live microorganisms (isolation and culturing) and whole microbial communities, collected and preserved suitably for molecular analysis.
- Provide protocols that deal with shipping requirements as well as shipping procedures that will ensure sample integrity and prompt arrival to the designated laboratory.

Laboratory microbiology is not part of the scope of this assignment. Relevant descriptive terminology will be provided to the Bioinformatics Committee.

Suggested Participants:
Kay Briggs (Chair), Bob Jonas, David Bourne, Rocco Cipriano, Pam Morris, Garriet Smith, John Argyle, Eugene Rosenburg, Emmett Shotts, Ginger Garrison, Debbie Santavy.

vii. Toxicology Committee

Responsibilities:

- Develop Standard Operating Procedures (SOP) which provide general method guidance; and
- Identify essential procedures for the collection, transport, processing, and analysis of coral samples for chemical content/contaminants.

Suggested Participants:
Marie Delorenzo (Chair), Jane Hawkridge, Geoff Scott, Stephen Klaine, Chip McCarty, Gary Rand.

viii. Bioinformatics Committee

Responsibilities:

- Organize a CDHC database that integrates standardized parameters and terminology used in data collection efforts and observations from the field and the laboratory. Selection and definition of these data have been tasked to the respective committees of the CDHC.
- Recommend and test appropriate data analysis methods to develop a better understanding of the factors associated with coral health and epidemiology.

Suggested Participants:
Laura Kracker (Chair), Kristy Larkin, Jonas Almeida, Colleen Charles, Sylvia Galloway, Jim Hendee.

ix. Molecular Committee

Responsibilities:

- Develop Standard Operating Procedures (SOP) that provide general method guidance; and
- Identify essential procedures for the collection, transport, processing and analysis of coral samples for cellular biochemicals, including DNA, RNA, and proteins.

Suggested Participants:
Charles Robinson (Chair), Craig Downs, Cheryl Woodley, Jo Ann Leong.

U.S. Department of Commerce
Donald L. Evans, Secretary

National Oceanic and Atmospheric Administration
Vice Admiral Conrad C. Lautenbacher, Jr., USN (Ret.)
Under Secretary for Oceans and Atmosphere