C. PATHOLOGY OF DISEASE

Identifying Diagnostic Tools Necessary to Adequately Characterize the Pathology Associated with Coral Disease

Background

Over the past three decades, coral reefs worldwide have experienced significant losses in living coral cover and changes in the structure and function of these communities. Infectious diseases have been recognized as a prominent cause of mortality in scleractinian corals in the western Atlantic since the 1980's, but until recently there were few reports of coral disease from the Indo-Pacific region. Current efforts to systematically assess the types and prevalence of coral disease in the Indo-Pacific suggest that coral disease also occurs commonly on Indo-Pacific reefs, and these diseases may have a greater role in structuring coral communities in the region than previously thought. Unfortunately, few diseases affecting Indo-Pacific corals have been adequately characterized, quantitative data on the spatial and temporal variability of diseases and their impacts are lacking for most locations, and linkages between environmental parameters and diseases affecting Indo-Pacific corals are unknown.

'Disease' is a word with many different connotations, depending on one's particular perspective or experiences. Coral biologists use disease almost exclusively to describe gross changes in a coral's appearance and usually assume that a disease is due to an infectious agent. Disease however, includes *"any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects; or combinations of these factors"* (Wobeser 1981). Therefore, as in other animal diseases, it is imperative that an unbiased approach is used when investigating coral disease.

Literally speaking pathology is the study (*logos*) of suffering (*pathos*); practically, it involves studying the structural and functional changes in cells, tissues and organs that define disease processes. There are four aspects that are investigated to understand disease processes: *etiology, pathogenesis, morphologic changes, and clinical significance* (Kumar et al. 2005). It is in this context that adopting concepts and principles of pathology will help to organize our observations and direct conclusions about coral disease in a rigorous and organized manner. Two recent publications address these issues for coral pathology (Work and Aeby 2006; Work et al. 2008b).

Etiology: its cause

Pathogenesis: mechanisms of disease development; sequence of events in response to etiologic agent

Morphologic changes: structural alterations in cells and organs

Clinical significance: functional consequences of the changes

Challenges and Recommendations:

The *Pathology of Diseases Working Group* (PDWG) was charged with identifying gaps in knowledge, research needs, and potential approaches that could be used to address pathology, pathogenesis and etiology of diseases in animals applicable to corals with appropriate modifications. Given the limited knowledge of the cause of many diseases in corals and the lack of uniformly applied methods to investigate disease, this topic was considered critical and timely.

The challenge for coral pathology is to develop approaches, procedural guidelines, and analytical methodologies that take advantage of the advances made in the study of the pathology and pathogenesis of disease in humans and other animals. There was general agreement within the group that the study of diseases in corals suffers from a lack of systematic investigation, particularly in regards to establishing case definitions and arriving at causality of disease. A case definition encompasses all the factors that define a particular disease, and can serve as a standardized point of reference for tracing the disease across different populations or geographic areas. Case definitions can change as new data on the particular disease appear. As a starting point, case definitions for newly described coral diseases should include good morphologic descriptions encompassing gross and microscopic pathology. More complete case definitions will include information about the causative agent and the pathogenesis of the disease. Many of the methods currently used to characterize diseases in terrestrial and other marine animals are applicable to corals.

For diseases that are novel or previously undescribed, carefully controlled laboratory studies can help elucidate the pathogenesis and cause of the disease. This includes studies evaluating host-agent interactions such as exposure of corals to suspected infectious or non-infectious agents in attempt to replicate clinical signs observed in the field. In cases where an etiologic agent is suspected to be necessary and sufficient to cause disease, this can be demonstrated through the use of Koch's postulates, where the animal is exposed to the isolated infectious agent, clinical signs reproduced, and the agent re-isolated from the animal (Work et al. 2008b). Unfortunately, Koch's postulates have often been applied to coral diseases based on identification of external characteristics (e.g., disease signs) without more detailed investigation of underlying cellular and structural characteristics of the experimentally reproduced lesion. These efforts have failed to distinguish between *primary* and *opportunistic* pathogens and have served only to enhance confusion in the literature. In addition, many diseases are complex making them difficult to study using Koch's postulates alone. Furthermore, many marine microorganisms are not culturable in laboratory settings thereby complicating their experimental manipulation. Through application of culture-independent methods, the presence of multiple disease-associated pathogens may be identified. In addition to traditional methods of morphological pathology (e.g. histopathology) and culture methods, the application of genomic, proteomic, and metabolomic-based approaches may be necessary to understand the pathology, pathogenesis and etiology of coral diseases.

General Recommendation: Develop key questions that might be asked in regards to a disease outbreak in corals.

At the outset, there was a consensus that great confusion existed on nomenclature of gross lesions in corals and that a good morphologic description provided a foundation for describing any disease. This led to considerable digression and discussion, however, in the end, the group decided on a decision tree that would give a broad outline on the process of disease investigations. In addition, three products were identified that were judged critical to sorting out existing knowledge gaps regarding disease in corals. These products included:

Key questions related to a disease outbreak

1. **How do you describe the disease?**
2. **Does it have a significant demographic effect?**
3. **Does it move rapidly?**
4. **Do corals recover?**
5. **Do you know what causes it?**
6. **Does it correlate with environmental factors?**

- Field identification cards for major lesions in corals from the Pacific.
- A summary of existing approaches to coral disease diagnostics.
- An approach to arrive at the suspected etiology of infectious disease in corals.

Strategic Objective C.1: Develop a decision tree for standardized investigation of coral diseases.

Recommendation C.1.1: The disease investigation process should follow a standard course of events.

An unusual mortality or morbidity event is signaled via presence of dead or dying corals (field signs) (Fig. C.1). Recognition of the event is followed by a systematic description of lesions (morphology) in affected corals leading to a decision point. Either a management decision is made (Management) based on field signs and gross morphology (e.g. continue observing, implement intervention, do nothing) or samples are taken for further laboratory diagnostics (Sample). The focus of laboratory diagnostics is to arrive at the cause of a lesion or to begin building a database of information that will add to the foundation of the case definition (morphology). If a causal agent is identified (or as information is accrued from the laboratory), this information is fed back to management. This communication has several purposes. First, it promotes "buy in" to the disease investigation on the part of management. Second, sharing of laboratory information with managers provides a forum for generation of further hypotheses and further sampling to arrive at cause of disease. Finally, if an etiology is identified, input from managers is critical in helping elucidate the ecology of the agent so that the disease can be effectively mitigated or potentially stamped out.

Strategic Objective C.2: Develop a field guide of common lesions observed in corals from the Indo-Pacific.

Recommendation C.2.1: A simple field guide to common lesions of corals in the Indo-Pacific should be developed, based on the approach identified for western Atlantic corals. This guide would include: accepted common name of lesion, morphologic description and representative photos (distance and macro).

Over the last 35 years coral reef researchers have identified and named over 50 diseases in scleractinian corals through field monitoring programs and targeted coral disease

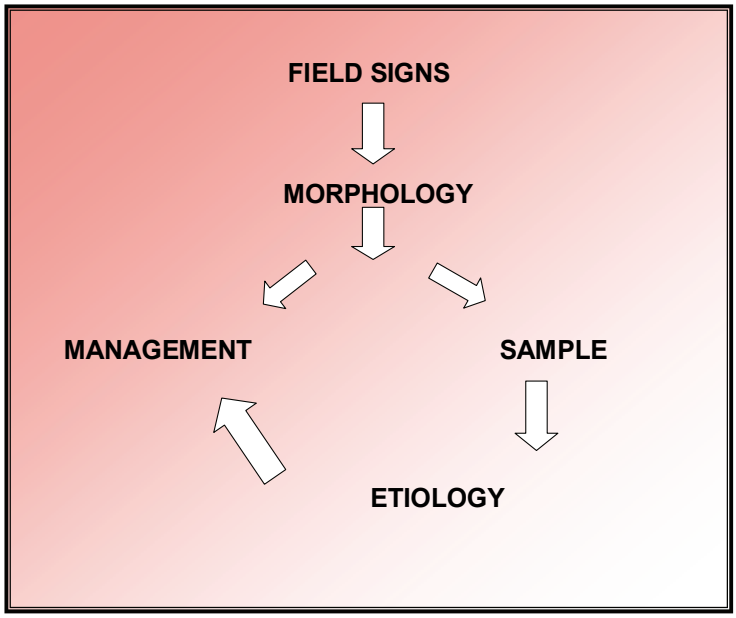

Fig. C.1 Disease Outbreak Response

research projects (Bruckner 2007; Green and Bruckner 2000; Weil 2004). While these observations have increased the visibility of coral diseases and have led to the recognition of the importance of coral diseases as a community structuring agent, the lack of a standardized approach to describe diseases has caused much confusion thereby limiting our ability to apply management tools in order to prevent disease occurrence and spread. Currently, coral disease is typically diagnosed in the field by identifying lesions, with comparative observations by different researchers and in different regions relying primarily on available photographs of gross signs and general descriptions based on the locations of lesions, the color of affected tissue or exposed skeleton, species affected, and rates of mortality. Unfortunately, this has led to a profusion of new names, including the use of different terminology to describe presumably similar gross field signs and similar terminology for syndromes observed in different ocean basins that have vastly different signs. Part of the problem has been that those describing coral diseases often infer causation based on gross appearance alone, however, the determination of causation is

something done more appropriately in a laboratory setting. Some progress has been made towards standardizing nomenclature of coral lesions (Work and Aeby 2006). More recently, the CDHC convened a workshop to establish diagnostic criteria (including nomenclature and case definitions) for coral syndromes affecting western Atlantic corals. Through this workshop, the CDHC developed a three-tiered approach to identify and differentiate coral diseases (Raymundo et al. 2008; Work and Aeby 2006)

Strategic Objective C.3: Review of existing laboratory methods to investigate coral diseases.

Recommendation C.3.1: Develop a white paper on investigative processes applied to coral diseases.

This review should include detailed information on pathology, microbiology (including bacteriology, virology, mycology and protozoology), toxicology, genomics/proteonomics and parasitology.

A variety of methods exist to investigate various aspects of coral disease, however, whether these methods are sufficiently standardized or adequate as currently applied remains questionable. The group recommended that a comprehensive literature search be implemented to review what methods have been used to investigate diseases of corals (from sampling to analysis), their limitations, and their potential.

Strategic Objective C.4: Identify a standardized approach to elucidate etiology of disease in corals.

Recommendation C.4.1: Assemble a model approach that could be used to determine whether a particular etiologic agent would have high probability of being associated with (or causal of) a lesion.

The model approach is based on answering certain critical questions:

Can a potential etiology be consistently visually associated with the lesion? For example, in some cases, an etiologic agent (bacterium, fungus, parasite or virus) is visibly associated with cellular damage either at the light or electron microscope level. Strong presumptions of causality can be inferred in cases where such findings are consistently associated with lesions.

Is the lesion transmissible? The group judged that transmission experiments could be done in the field (and also the lab) under the following conditions:

- These are limited to a restricted geographic area (currently unspecified but probably carried out in the immediate area such as a 0.5 km radius).
- That healthy fragments be attached to diseased colonies (and not vice versa).
- That appropriate controls be run for all transmission experiments.

[**Editor's note:** In the final plenary session of the Workshop, the participants of all the working groups discussed field transmission experiments and decided they should be put

on hold until specific guidelines could be developed. See the OPINION paper by Cheryl Woodley in Appendix VI.]

The illustration below (Fig. C.2) shows the general concept in determining the nature of a communicable agent. If field or laboratory trials indicate the lesion to be communicable, subsequent experiments are moved into the laboratory. There, tissues from the diseased corals are extracted (methods vary), filtered or unfiltered extracts are inoculated onto susceptible colonies, and those observed for development of lesions. If lesions are reproduced using 0.1µm filterable extracts, it is assumed that causative agent is subcellular element (virus, protein, nucleic acid or chemical). If lesions are reproduced using non-filtered extracts, it is assumed that causative agent is cellular (e.g. bacteria, parasite). A more comprehensive schema and approach is available elsewhere (Work et al. 2008b)

For non-filterable agents, clues can sometimes be gained by visual association (e.g., light or electron microscopy) as to its identity. In many cases, however, there are too few organisms to visualize effectively, and attempts must be made to culture. Although many bacteria in the marine environment are not culturable, attempts should be made to rule out culturable bacteria by using a variety of selective and non-selective media (including anaerobic conditions) to compare flora between sick and healthy individuals in efforts to target potential organisms in trials to fulfill Koch's postulates. Genomic approaches can also be used to compare sick and healthy corals; however, because of the large number and variety of organisms detected using these methods, large samples sizes may be necessary and this approach, though helpful in generating hypothesis, rarely gives a definitive cause of the lesion. Methods to detect culture-independent flora associated with corals are still under development and will continue to develop. In addition, there is a need to develop primers to detect bacteria associated with corals.

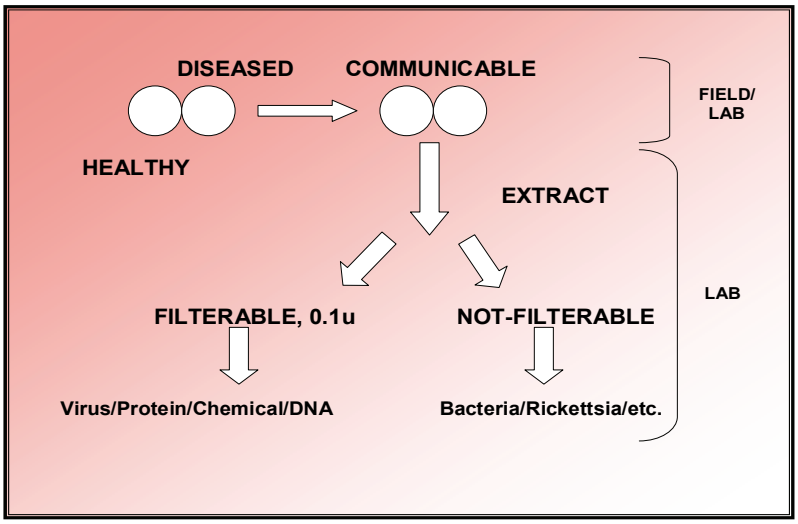

Fig. C.2 Scheme for Determining the Nature of a Communicable Disease

Standard methods exist to identify filterable agents such as viruses. These include electron microscopy, sucrose density gradients (physical separation based on density), degenerate primers and PCR, and susceptibility to chloroform or ether (to assess whether enveloped or unenveloped particles are present). A major limitation to the study of virology in corals is the current lack of laboratory cell culture systems (see Recommendation 3.2 in the PDWG section indicating the need for coral cell lines). Standard methods also exist to identify filterable agents that are not viruses. Extracts can be treated with chloroform and the aqueous and lipid soluble phase assayed for effect (lesion) in corals. Such methods coupled with gas chromatography-mass spectrometry can help identify compounds (chemicals) that may be associated with presence of lesions.

Strategic Objective C.5: Build critical scientific capacity in the field of coral pathology and offer a health management perspective in resource management.

Recommendation C.5.1: Create and Support Advanced Educational Opportunities.

Strategic Objective C.6: Improve capacity to Manage Coral Disease Outbreaks.

Recommendation C.6.1: Establish a Coral Disease Outbreak and Unusual Mortality Response Program.

A response program should be developed that involves a National Center that provides guidance in responding to disease outbreaks and serves as a repository for information, regional coordinators and local responders. The National Center should organize training programs for Response Teams in strategic Pacific and Caribbean locations and whenever possible should assist in the investigation of coral disease outbreaks, facilitate processing of samples, and ensure relevant results and recommendations are provided to resource managers, participants and stakeholders in a timely manner. The National Center should also develop, with input from experts, a manual with a set of tools and procedures for investigating coral disease.

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