I. Executive Summary

Scleractinian (hard) corals appear to be increasingly susceptible to pathogenic diseases, yet it is poorly understood why certain individuals, populations or species are more susceptible to diseases than are others. An understanding of mechanisms of disease resistance in corals is essential to our understanding of patterns of disease incidence and virulence. Recent studies indicate that coral diseases are prevalent on Hawaiian reefs and have demonstrated variability in susceptibility to disease at both the species and population level. Thus, the study of mechanisms of resistance to disease in Hawaiian corals is particularly timely. This research program will examine chemical resistance as a potential mechanism of immune function for corals against pathogenic microbial infections. Specific objectives of this proposal will include: (1) determining differences in antimicrobial activity among the dominant coral genera in Hawaii, (2) assessing host-specific (population level) variability in antimicrobial activity of common coral species from different sites on Oahu, and (3) assessing differences in antimicrobial activity and chemical constituents in healthy vs. infected colonies of the same species. The first and second objectives will provide resource managers with information regarding the relative susceptibility of specific coral genera and populations to newly emerging or invading pathogens, and will enable them to make decisions regarding levels of future monitoring and management. The third objective will examine variability in susceptibility to disease at the level of the individual coral colony and will provide insights as to why certain colonies acquire an infection even if their neighbors do not. This information will help explain patterns of disease incidence on the reef and can be used by coral reef managers to select resistant coral genotypes for transplant or other restoration projects on impacted reefs.

III. Purpose

A. Detailed description of the resource management problem(s) to be addressed.

Management of disease in wildlife populations has been accomplished by reducing or removing the source of infection or reducing the spread of disease. However, before management actions can be developed, there needs to be a basic knowledge of disease dynamics. Relatively little is known about the epizootiology of coral disease and our project provided information as to why levels of disease may vary among and within coral populations.

B. Detailed description of the question(s) asked to answer the resource management problem(s)

We addressed the question of whether or not common Hawaiian corals used chemical resistance
as a mechanism of defense against microbial infections. Levels of disease vary between Hawaiian coral genera and among sites surveyed. We examined whether differences in antimicrobial defenses within and among corals could help explain these patterns. We also looked for induced chemical defenses in diseased colonies. Knowledge such as this allows managers to tease out whether differences in disease levels among sites or corals may be due to environmental stressors vs. differences in chemical defenses within and among coral populations.

C. Objectives to answer each question.

Goal 1 was to assess differences in antimicrobial activity among the different coral genera in Hawaii.
Goal 2 was to assess host-specific (population-level) variability in antimicrobial activity of common coral species from different sites on Oahu.
Goal 3 was to assess differences in antimicrobial activity and chemical constituents in healthy vs. infected coral colonies.

IV. Approach
Detailed description of the work performed for each objective from III(C), including (but not limited to):

Goal 1 (assess differences in antimicrobial activity among the different coral genera in Hawaii)

A. list individuals and organizations actually performing the work

D. Gochfeld oversaw method development of the bacterial growth and cytotoxicity assays, trained students in sample processing, oversaw running the assays, and processed and analyzed the data. J. Miller performed the method development and trouble shooting of the assays. G. Aeby and her students collected the coral samples and shipped them to the University of Mississippi. A. Katzenmeyer and S. Bailey extracted the samples and ran the assays.

B. material list

Collection (Ziplocs, hammer, chisel, cooler)
Extraction (beakers, solvents, vials, freezer, lyophilizer, speedvac, balance, hot plate, wax)
Bacterial growth assays (bacterial strains from ATCC and DSMZ, culture flasks, various growth media, 96-well plates, multi-channel pipettor, incubator with shaker, plate reader)
Cytotoxicity growth assays (pipettor, petri plates, various growth media, incubator)

C. construction instructions for anything used to accomplish the III(C) objectives

NA

D. deployment steps

NA

E. data collection procedures
Bacterial strains were incubated in media or media amended with extract for 5-24 hours depending upon strain. Optical density was read initially and at the end of the assay using a Bio-Tek Plate Reader. Wells with negative growth curves were plated onto media and incubated for 24 hours, and growth or no growth was recorded.

F. data analysis techniques

Differences among coral genera were analyzed by comparing the slopes of the growth curves against each bacterial strain using one-way ANOVAs.

G. photos from research during each stage (construction, in situ, lab)

<table>
<thead>
<tr>
<th>Species studied</th>
<th>96-well plate for bacterial growth assay:</th>
<th>Extract with cytotoxicity:</th>
<th>Extract with Cytostatic activity:</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pocillopora meandrina</em></td>
<td><img src="path/to/image" alt="image" /></td>
<td><img src="path/to/image" alt="image" /></td>
<td><img src="path/to/image" alt="image" /></td>
</tr>
<tr>
<td><em>Porites lobata</em></td>
<td><img src="path/to/image" alt="image" /></td>
<td><img src="path/to/image" alt="image" /></td>
<td><img src="path/to/image" alt="image" /></td>
</tr>
<tr>
<td><em>Montipora capitata</em></td>
<td><img src="path/to/image" alt="image" /></td>
<td><img src="path/to/image" alt="image" /></td>
<td><img src="path/to/image" alt="image" /></td>
</tr>
</tbody>
</table>

H. contact information for companies used to purchase items unique to your project

NA

Goal 2 (assess host-specific (population-level) variability in antimicrobial activity of common coral species from different sites on Oahu)

I. list individuals and organizations actually performing the work

Same as Goal 1

J. material list
K. construction instructions for anything used to accomplish the III(C) objectives
NA
L. deployment steps
NA
M. data collection procedures
Same as Goal 1
N. data analysis techniques

Differences among sites were analyzed by comparing the slopes of the growth curves against each bacterial strain using one-way ANOVAs.

O. photos from research during each stage (construction, in situ, lab)
Same as Goal 1

P. contact information for companies used to purchase items unique to your project
NA

Goal 3 (assess differences in antimicrobial activity and chemical constituents in healthy vs. infected coral colonies)

Q. list individuals and organizations actually performing the work

Same as Goal 1 through the assay phase. Following the assays, A. Katzenmeyer prepared samples for chemical fingerprinting, and D. Gochfeld performed the method development for the chemical fingerprinting and processed and analyzed the data.

R. material list

Same as Goal 1 through the assay phase.
Chemical fingerprinting (HPLC vials with inserts, Phenomenex Synergi-Hydro column, solvents, Waters HPLC)

S. construction instructions for anything used to accomplish the III(C) objectives
NA
T. deployment steps
NA
U. data collection procedures
Same as Goal 1 for the assay phase. A small amount of extract from the healthy, diseased and
control *Montipora capitata* samples was dissolved to known concentration and injected into an analytical HPLC. This yielded a chromatography spectrum and the peaks were integrated to yield areas under the curve. Major peaks were included in the analysis.

V. data analysis techniques

Differences among coral condition (healthy, diseased, control) were analyzed by comparing the slopes of the growth curves against each bacterial strain using one-way ANOVAs. Areas under the curve from the chemical fingerprints were entered into excel and one-way ANOVAs were performed on the areas of each peak (n=22) with coral condition as the factor and colony as the replicate.

W. photos from research during each stage (construction, in situ, lab)

Same as Goal 1

X. contact information for companies used to purchase items unique to your project

NA

VI. Results

A. Findings for each III(C) objective.

Objective 1: Overall, corals from three dominant Hawaiian coral genera exhibit a high degree of antimicrobial activity (Fig. 1), and this varies among coral genera (Fig. 2). All three coral species exhibited selectivity in their antimicrobial activity against certain bacterial strains (Fig. 2), with overall high levels of defense against certain bacteria and low levels of defense or even stimulatory activity towards other bacteria (Fig. 2). Clearly Hawaiian corals produce antimicrobial chemical defenses, but their defenses are highly selective for different types of bacteria.

![Graph showing antimicrobial activity](image)

**Figure 1.** Overall antimicrobial activity among Hawaiian corals.
### Table 1. Intraspecific variability in antimicrobial activity of Hawaiian corals from three sites on Oahu. Numbers are extracts (out of n=5 per assay) with inhibitory (negative) or stimulatory (positive) effects on each bacterial strain.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Porites lobata</th>
<th>Pocillopora meandrina</th>
<th>Montipora capitata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Waianae</td>
<td>Maile Beach</td>
<td>Kaneohe Bay</td>
</tr>
<tr>
<td>Aurantimonas coralicida</td>
<td>-3</td>
<td>-4</td>
<td>-5</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>-4</td>
<td>-5</td>
<td>-5</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>+2</td>
<td>-1</td>
<td>+1</td>
</tr>
<tr>
<td>Pseudomonas nautica</td>
<td>+5</td>
<td>+3</td>
<td>+5</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>-5</td>
<td>-5</td>
<td>-5</td>
</tr>
<tr>
<td>Vibrio agarivorans</td>
<td>-5</td>
<td>-5</td>
<td>-5</td>
</tr>
<tr>
<td>Vibrio corallyticus</td>
<td>+3</td>
<td>-5</td>
<td>-5</td>
</tr>
<tr>
<td>Vibrio shiloi</td>
<td>-3/+1</td>
<td>-1/+1</td>
<td>-1/+1</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>-5</td>
<td>-5</td>
<td>0</td>
</tr>
<tr>
<td>Overall % activity</td>
<td>55.6</td>
<td>68.9</td>
<td>57.8</td>
</tr>
</tbody>
</table>

**Objective 2.** All three genera of Hawaiian corals exhibited intraspecific variability both within and between populations (Tables 1). This may, in part, explain why we see a high degree of variability in disease prevalence both among and within sites.
healthy tissues on colonies of *Montipora capitata* affected by *Montipora* white syndrome along with their nearest neighbor healthy (control) colonies. There was significantly greater antimicrobial activity in control corals than in healthy tissues on affected corals against *Yersinia enterocolitica* and a trend towards higher antimicrobial activity against several other bacterial strains (Fig. 3), suggesting that higher levels of antimicrobial activity in control corals may reduce their susceptibility to disease. This variability in defenses may explain why diseased corals on the reef may occur adjacent to unaffected corals. There was also a significantly higher level of antimicrobial activity in the diseased tissues than in the healthy tissues on affected colonies against *Aurantimonas coralica*, and a trend towards higher antimicrobial activity against several other bacterial strains (Fig. 4), suggesting that antimicrobial defenses may be induced in diseased tissues in an attempt to fight off the infection.

![Graph showing antimicrobial activity comparison](image)

**Fig. 3.** Comparison of antimicrobial activity in control (healthy) colonies and healthy tissues on diseased colonies. The greater the negative slope, the greater the antimicrobial activity.
Fig. 4. Comparison of antimicrobial activity between healthy and diseased tissues on corals affected with *Montipora* white syndrome.

Chemical fingerprints of the control, healthy and diseased extracts were compared to examine differences in chemical profiles. In general, the profiles were very similar (Fig. 5), but two peaks had significantly greater concentrations in the control corals than in the healthy tissues on the diseased corals, and a third peak showed a trend in this direction (Fig. 6A). Those same three peaks showed trends toward greater concentrations in the diseased tissues than in the healthy tissues of the affected corals (Fig. 6B). These peaks may represent the antimicrobial compounds responsible for the differences in activity seen among the control and healthy extracts, and they may contribute to an induced defense in the diseased tissue. Further research is needed to tease apart these patterns.
Hawaiian corals are capable of antimicrobial defense and there is variability among and within coral populations. Healthy colonies of *Montipora capitata* had higher levels of antimicrobial activity than did infected colonies and infected regions of colonies with *Montipora* white syndrome had higher levels of activity compared to the healthy regions. These results all offer partial explanations for the patterns of disease occurrence that are found on the reefs of Hawaii.

C. Site specific results for each location (Can place in an appendix as electronic file). See Table 1
VII. Resource Management Implications
   A. Given the results from VI, what are the implications for resource managers?

   Managers must make decisions about how to best protect their resources. Part of that decision-making depends on the ability to accurately assess and interpret patterns of coral health in the field. Based on our findings we now know that coral’s do have defense mechanisms against bacterial infections and these levels of protection can vary between and within coral populations.

   B. How do these implications and results help to address the resource management problem(s) identified in III(A)?

   This project provided basic information on mechanisms of defense by coral against bacterial infections.

   C. What recommendations for resource managers can be made based on the implications and results?

   We recommend that the variable antimicrobial defense mechanisms of coral be taken into consideration when interpreting patterns of coral health in the field. The next step will be to determine how various stressors affect a coral’s ability to produce the antimicrobial compounds and thus affect their ability to withstand disease.

VIII. Evaluation
   For each III(C) objective:
   A. Describe the extent to which the objective was attained, including:
      1. Was the objective attained? How? If not, why?

   We did attain each objective, as described above.

      2. Were modifications made to objective? If so, explain.

   We originally proposed to perform attachment assays in addition to bacterial growth assays, however, chemical nature of the extracts precluded this. Several methods were attempted, however all attachment assays are based upon detecting the bacteria using various wavelength-dependent strains (e.g., UV). The compounds within our extracts all absorb at the wavelengths in question and therefore we were unable to visualize the bacteria as the signal from the extract itself masked them and we were unable to obtain results.

      3. If significant problems developed, resulting in less than satisfactory or negative results, discuss.

   See above for attachment assays. All other results were satisfactory.

      4. Description of need, if any, for additional work.
We completed the object, specified within this proposal. Future work will focus on teasing apart the broad patterns that we identified. We plan to examine population-specific variability on a more focused level – that is, by comparing sites with known differences in disease prevalence and sites with known differences in certain stressors, as stressors are known to impact the ability of organisms to produce chemical defenses. We also plan to characterize the chemical defense and further elucidate patterns of defense induction in response to disease, by performing temporal and spatial sampling on known affected colonies to determine the timing and scale of the induction. Once specific pathogens of Hawaiian coral diseases have been identified, we will reevaluate these corals to determine whether patterns of resistance to specific pathogens can explain patterns of prevalence of that disease. We will also evaluate how different stressors may affect a coral’s ability to produce antimicrobial compounds and thus affect their disease susceptibility.

B. What performance measures are used to evaluate how well the project met the stated objective?

Performance measures include completion of stated objectives, analysis of results, production of reports and presentations.

IX. Dissemination of Project results:

A. Explain, in detail, how the projects results have been, and will be, disseminated.

Results have been presented at HCRI meetings in January 2006, May 2006, August 2006 and January 2007. Results will be presented at scientific meetings and a publication will be prepared and submitted for publication in a scientific journal.

B. List of publications, workshops, and presentations


C. Data or information products

Two quarterly and a final report have been produced, four presentations made during HCRI meetings, and one poster presentation of results at a national meeting. These results will also be presented at a national meeting in March 2007 and a peer-reviewed publication is in preparation.

D. Partnerships established with agencies or organizations

This project marks the first collaboration between the University of Mississippi and the Hawaii Institute of Marine Biology.