

State of Hawai'i
DEPARTMENT OF LAND AND NATURAL RESOURCES
Division of Aquatic Resources
Honolulu, Hawai'i 96813

July 27, 2007

Board of Land
and Natural Resources
Honolulu, Hawai'i

Conditional Request for Authorization and Approval to Issue a Papahānaumokuākea Marine National Monument Research Permit to Dr. Greta Aeby, University of Hawai'i, Hawai'i Institute of Marine Biology (HIMB), for Access to State Waters to Conduct Coral and Fish Disease Research Activities.

The Division of Aquatic Resources (DAR) hereby submits a conditional request¹ for your authorization and approval for issuance of a Papahānaumokuākea Marine National Monument (PMNM) research permit to Dr. Greta Aeby, HIMB, pursuant to § 13-60.5, *Hawaii Administrative Rules*, and § 187A-6, *Hawaii Revised Statutes*, and all other applicable laws and regulations. The research permit, described below, will allow activity to occur in the NWHI State Marine Refuge (0-3 miles) waters surrounding Nihoa Island, French Frigate Shoals, Gardner Pinnacles, Laysan Island, Lisianski Island, Pearl and Hermes Atoll, and Kure Atoll. The activities will occur from August 13, 2007 through September 30, 2007, as outlined below and in the attached permit application.

INTENDED ACTIVITIES

The applicant proposes to measure changes in coral disease levels through time, measure rates of tissue loss from different diseases, determine patterns of disease transmission among colonies, and measure the rate of spread of disease and evaluate changes in coral cover and coral species composition.

Objectives:

- 1) To re-survey permanent sites at FFS, Pearl and Hermes, Midway and Kure established in 2005 for the assessment of disease dynamics.
- 2) To survey additional *Acropora* rich sites within FFS.
- 3) Determine the etiology of *Acropora* wasting syndrome (AWS).
- 4) Determine whether AWS is infectious .
- 5) Determine the affect of *Acropora* growth anomalies on the growth of table corals.
- 6) Examine ta'ape (*Lutjanus kasmira*) and native goatfishes (*Mulloidichthys flavolineatus*, *Parupeneus multifasciatus*, *P. porphyreus*) for presence of disease.

¹ Because there is an administrative enforcement action alleging that Dr. Aeby violated her 2006 permit pending before the Board, DAR staff's request for approval of this permit is made with an understanding that it is conditioned upon the Board's decision on the enforcement action. See "Recommendations" section, below, for a more thorough discussion of this issue.

7) Examine the surgeonfish, *Ctenochaetus strigosa*, affected by pigmentation disease.

PROCEDURES

Disease Studies

Re-survey of established sites at FFS, Pearl and Hermes (PHR), Midway (MID) and Kure (KUR) will follow established protocol. Two 25 m lines will be laid out along the substrate. A diver will then swim over the lines during which all corals within one half meter of either side of the transect lines will be identified to species, counted, and assigned to a size class. In the same manner, a second diver will swim over the lines and examine all corals for signs of bleaching or disease. Bleached colonies will be assigned a bleaching category: 0-no bleaching; 1- 10-30%; 2- 30-50%; 3-50-100%; 4-50-100%; 5-mortality. For corals exhibiting disease, a general description of the condition will be recorded, the coral will be photographed and a specimen will be collected for histopathological examination.

Growth Studies

Colonies of *Acropora cytherea* with growth anomalies and a nearest neighbor of similar size will be measured (length and width of each tier), photographed (with a ruler) and tagged. They will be re-examined the following year (2008) to look for differences in growth between affected and control colonies. The applicant will be tagging colonies located at their established permanent sites at FFS.

Disease Etiology

Note – Dr. Aeby has withdrawn her request to transport frozen live cultures of healthy and diseased coral to O‘ahu.

Small fragments of infected coral and healthy fragments for control will be collected at depth and placed into individual bags. Samples will be held on ice and transported to the ship where they will be processed. To determine whether the disease is infectious, a subsample of the fragments will first be used for transmission studies. An infected fragment and a control fragment will be held together in a bucket of seawater for 24-48 hours. The buckets will have tight fitting lids with a small hole for an air line and will be placed in tubs of fresh water providing secondary containment. The tubs will be secured in a protected area on deck. After a maximum of 2 days, the coral fragments will be examined, photographed and processed crushing with a sterile mortar and pestle and mixing with 10ml of sterile seawater. The mixture will be placed in sterile tubes in 15% glycerol stock solution and frozen at -80C. This procedure results in frozen microflora which will be re-cultured back on O‘ahu to conduct infection trials at a secure PC2 facility. Any seawater or equipment exposed to infected coral will be sterilized with 10% bleach solution which is a standard sterilization technique used in pathogen studies. To ensure that the transmission study is completed before leaving FFS, the study will be initiated immediately upon arrival at FFS. In addition, the chief scientist will be notified of the start and end of all trials so that can be taken into account if cruise schedules need to be adjusted.

Non-lethal Environmental Screening

To investigate the potential use of non-lethal environmental screening, the applicant proposes to collect seawater and sediment adjacent to infected colonies and swab or syringe the mucus from infected and healthy colonies. Samples will be collected in the field, held on ice and transported to the ship where they will be frozen for later molecular analysis. At HIMB, DNA will be extracted, amplified and microorganisms identified.

Fish Disease Screening

Target species of fish will be collected by spear, placed on ice and transported to the ship for examination. Fish will be weighed and measured (standard and fork length), examined systematically externally and internally, and gross lesions documented. For histopathology, sections of skeletal muscle, skin, spleen, liver, cranial and caudal kidneys, swim bladder, brain, heart, gill, and gonad, small intestines, and stomach will be excised and fixed in 10% neutral buffered formalin. Tissues will be sectioned, dehydrated in alcohol series, embedded in paraffin, sectioned at 5 μm , placed on microscope slides, stained with hematoxylin and eosin, and examined using a microscope.

REVIEW PROCESS:

The permit application was sent out for review and comment to the following scientific entities: Division of Aquatic Resources staff, Papahānaumokuākea Marine National Monument, NOAA Pacific Islands Regional Office (NOAA-PIRO), and United States Fish and Wildlife Service. The Office of Hawaiian Affairs (OHA), and the Kahoolawe Island Reserve Commission (KIRC) were also consulted.

Comments received from the scientific community are summarized as follows:

1. If Hawaiian Monk Seals are in the area while performing any transect, camera drop, SCUBA operations, fishing activity, or sampling activity, the activity shall cease until the animal(s) depart the area.
2. The applicant and those listed on the permit shall view and be familiar with “marine Wildlife Viewing Guidelines” available at <http://www.nmfs.noaa.gov/pr/education/viewing.htm>
3. Applicants abide by new Disease Protocols.
4. Is any provision made for preventing possible contamination of healthy corals by implements (e.g. chisels) used to sample diseases corals? (Note – this was received from a reviewer unfamiliar with the Disease and Transport Protocols).

Comments received from the Native Hawaiian community are summarized as follows:

1. OHA requests that the research vessel have at least one cultural practitioner on board.
2. OHA urges the State to find all applications incomplete (and therefore will not be processed) if they do not include a reference to cultural research, consultation, and resulting protocol.

3. One reviewer requested that once a coral specimen is no longer required, that it be transferred to a Native Hawaiian Organization for a culturally appropriate method of disposal.

RESPONSE:

A meeting was held between HIMB researchers and administrators, and DAR staff, to discuss reviewer's (Scientific, Policy, and Cultural) concerns. The concerns raised by OHA were also discussed at the meeting. All applicants agreed to abide by the three comments received from the scientific community. Scientific comment number four (4) is addressed in Final Staff Recommendation four (4) below.

Additionally, it was the consensus of those in attendance, that in order to address the requests from OHA, more information is required from OHA. Specifically, a list of acknowledged cultural practitioners who are available to accompany specific research cruises should be provided by OHA; additionally, a briefing for science researchers should be arranged by OHA or a designate, to provide the information needed by individual applicants in order to address OHA's request for cultural impact analysis.

IMPACT ANALYSIS

Although some disturbances to Monument resources may be necessary to achieve the objective of this project, this research should have minimal impact on Monument resources. The effective management of marine protected areas requires detailed information regarding disease among various populations of species. This information is needed by the Co-Trustees to effectively manage the resources of the Monument. Therefore this study is important in defining the relevant units of management for the Monument and to assess the degree to which coral disease and is present and rate at which it may spread in the Monument. Therefore, the value of this research far outweighs the minimal effects of the research activities.

FINAL STAFF RECOMMENDATIONS:

DAR staff is of the opinion that the Applicant has properly demonstrated valid scientific justification for her application. Additionally, DAR staff feels that the research proposed in Dr. Aeby's application is important for the continuation of a long-term coral disease monitoring program that has been ongoing since 2002 at these sites, and the results of this research are important for effective Monument management. The PMNM Monument Management Board concurs with this opinion.

If approved by the Board, the applicant may be allowed to enter the NWHI State waters and to conduct the activities therein as specified in the application with the following special instructions and conditions, which are in addition to the Papahānaumokuākea Marine National Monument Research Permit General Conditions:

1. Require Applicant to inform and consult with DAR regarding their cruise plan before each trip to the NWHI.
2. Research operations must cease if monk seals are present in the immediate vicinity.

3. No fishing is allowed in State Waters except as authorized under State law for subsistence, traditional, and customary practices by Native Hawaiians.
4. To prevent introduction of disease or the unintended transport of live organisms, the permittee must comply with the disease and transport protocol attached to this permit.
5. Tender and dive vessels operating within the Monument are encouraged to operate at slow speed and with a bow lookout in shallow water coral reef areas in order to minimize prop or bow damage to three dimensional coral reef habitat or endangered monk seals or sea turtles.
6. Tenders and small vessels must be equipped with engines that meet EPA emissions requirements.
7. Refueling of tenders and all small vessels must be done at the NOAA ship Hi'ialakai and outside the confines of the lagoons or near-shore waters.
8. This permit is not to be used for nor does it authorize the sale of collected organisms. Under this permit, the authorized research activity, including work involving a bioassay or bioprospecting, must be for non-commercial purposes, i.e., not involving the use or sale of any organisms, byproducts, or material collected within the Monument for obtaining patents or intellectual property rights for profit.
9. The permittee may not convey, transfer, or distribute, in any fashion (including, but not limited to, selling, trading, giving, or loaning) any coral, live rock, or organism collected under this permit without the express written permission of the Co-Trustees.

RECOMMENDATION:

HAR §13-60.5-6 requires the Board to consider whether the applicant has violated or not complied with any term or condition of previous permits issued by the board in evaluating any permit application. The pending enforcement action alleging that Dr. Aeby violated her 2006 permit, and the Board's decision in that enforcement action, must be considered in evaluating Dr. Aeby's current permit application. The recommendation of the Department depends on the Board's decision in the enforcement action. As such, the Department is making the following alternative recommendations:

1. If the Board has found that Dr. Aeby violated her 2006 permit, then, in consideration of the finding of a violation of a prior permit and pursuant to HAR §13-60.5-6(a)(3), the Board must deny Dr. Aeby's permit application.
2. If the Board does not find that Dr. Aeby violated her 2006 permit, then the Board may approve the issuance of a 2007 permit to Dr. Aeby based on the factors set out in HAR § 13-60.5-6(a)(4).² Based on the scientific review contained herein, DAR staff believes

² HAR § 13-60.5-6(a)(4) states: "[a]pproval may be granted by the board after an assessment of the appropriateness of the activity described in the application based on: (i) Factors that the board and department consider relevant to section 13-60.5-1, the intent and purpose of this chapter, and (ii) All applicable state and federal laws, including written proof of compliance with the federal permit

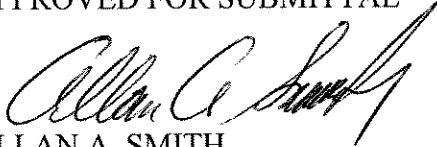
that Dr. Aeby's research is valuable to the management and conservation of the Papahānaumokuākea Marine National Monument and therefore recommend the issuance of a 2007 permit to Dr. Aeby.

Respectfully submitted,



DAN POLHEMUS
Administrator

APPROVED FOR SUBMITTAL



ALLAN A. SMITH
Interim Chairperson

requirements governing commercial and recreational fishing in adjacent waters. (iii) Submission of an application or the receipt of public comments on the application shall not obligate the board to issue a permit or constitute a right on the part of an applicant to receive a permit.”



Department of Land and Natural Resources
Northwestern Hawaiian Islands Permit Application Review

Permit Type: Management Recreation Research
Education Cultural Special Ocean Use

Working Title: Request for Authorization and Approval to Issue a Papahānaumokuākea Marine National Monument Research Permit to Dr. Greta Aeby, University of Hawai‘i, Hawai‘i Institute of Marine Biology (HIMB), for Access to State Waters to Conduct Coral and Fish Disease Research Activities.

Project Applicant: Dr. Greta Aeby, HIMB

Project Location(s) (Both State Waters and Monument): Nihoa Island, French Frigate Shoals, Gardner Pinnacles, Laysan Island, Lisianski Island, Pearl and Hermes Atoll, and Kure Atoll.

Project Dates and Duration: August 13, 2007 through September 30, 2007

Project Précis & Background (Summary of project and why this is proposed):

The proposed research is a genetic survey of reef fishes, designed to address the issue of population connectivity across the NWHI. The applicant is currently surveying 25-30 species across the entire archipelago, using mitochondrial DNA (mtDNA) sequencing technology. With a few notable exceptions, reef organisms have not been surveyed on this scale.

Are there other relevant permits that have/will be issued with regard to this project? No

What is the relevance to management and/or the improved understanding of NWHI & MHI?

The effective management of marine protected areas requires detailed information regarding disease among various populations of species. This information is needed by the Co-Trustees to effectively manage the resources of the Monument. Therefore this study is important in defining the relevant units of management for the Monument and to assess the degree to which coral disease and is present and rate at which it may spread in the Monument.

Could work be conducted outside the NWHI?: Yes No

Has Applicant been granted a permit from the State in the past? Yes

If so, please summarize past permits:

Permit No. DLNR.NWHI06R008 was issued to the applicant in 2006 for similar research

Have there been any a) violations: Yes No **b) late/ incomplete reports:** Yes No

Any other relevant concerns from previous permits? Dr. Aeby is currently under investigation for possible permit violations in 2006.

Recommendations:

DAR Staff: Defers to the State of Hawai'i, Board of Land and Natural Resources for the decision.

NH CWG: Approve this permit application Reject this permit application

Additional Comments:

Northwestern Hawaiian Islands Marine National Monument
Permit Application

NOTE: This Permit Application (and associated Instructions) are for activities to be conducted in the Northwestern Hawaiian Islands Marine National Monument, including Hawaiian Islands National Wildlife Refuge, the Midway Atoll National Wildlife Refuge, Battle of Midway National Memorial, Northwestern Hawaiian Islands State Marine Refuge, Kure Atoll Hawaii State Seabird Sanctuary, and the Northwestern Hawaiian Islands Coral Reef Ecosystem Reserve. The Co-Trustees are required to determine that issuing the requested permit is compatible with the findings of Presidential Proclamation 8031. Within this Application, please provide all information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historical and cultural resources of the NWHI Marine National Monument (Monument).

Summary Information

Applicant name: Greta S. Aeby

Permit categories:

- Research – Please fill out Sections A-D (as applicable) and Appendix A
- Conservation and Management - Please fill out Sections A-D (as applicable) and Appendix A
- Education - Please fill out Sections A-D (as applicable) and Appendix B
- Native Hawaiian Practices - Please fill out Sections A-D (as applicable) and Appendix C
- Recreation (Midway ONLY) - Please fill out Sections A-D (as applicable) and Appendix D
- Special Ocean Use - Please fill out Sections A-D (as applicable) and Appendix E

Briefly describe permit activity:

- This application is for a RENEWAL of an existing permitted project.
- This application is for a NEW project.

When will the activity take place?

From: August 1 To: August 25, 2007

NOTE: INCOMPLETE APPLICATIONS WILL NOT BE CONSIDERED

Please Send Permit Applications to:

NWHI Marine National Monument Permit Coordinator

6600 Kalaniana'ole Hwy. # 300

Honolulu, HI 96825

Hoku.johnson@noaa.gov

PHONE: (808) 397-2660 FAX: (808) 397-2662

NOTE: SUBMITTAL VIA ELECTRONIC MAIL IS PREFERRED BUT NOT REQUIRED. FOR ADDITIONAL SUBMITTAL INSTRUCTIONS, PLEASE SEE PG 7.

Section A - Applicant Information

1. Applicant

Name (last, first, middle initial): Aeby, Greta S.

Title: Assistant researcher

2. Mailing address (street/P.O. box, city, state, country, zip):

44-138 Kahinani Way, Kaneohe, HI, USA 96744

Phone:808-386-4784

Fax:808-236-7443

Email:greta@hawaii.edu

For students, major professor's name, telephone and email address:

3. Affiliation (institution/agency/organization directly related to the proposed project):

Hawaii Institute of Marine Biology

4. Additional persons to be covered by permit: Thierry Work, USGS, 792-9520, thierry_work@usgs.gov; Megan Colvin, UH, 236-7437, colmu_tgww@hotmail.com, Meir Sussman, Australian Institute of Marine Science, meir.sussman@jcu.edu.au, other personel to be determined

Section B: Project Information

5a. Project location(s):

- | | | |
|-------------------------------------------------------|-------------------------------------|--------------------------------------|
| <input type="checkbox"/> Nihoa Island | <input type="checkbox"/> Land-based | <input type="checkbox"/> Ocean-based |
| <input type="checkbox"/> Necker Island (Mokumanamana) | <input type="checkbox"/> Land-based | <input type="checkbox"/> Ocean-based |
| x French Frigate Shoals | <input type="checkbox"/> Land-based | x Ocean-based |
| <input type="checkbox"/> Gardner Pinnacles | <input type="checkbox"/> Land-based | <input type="checkbox"/> Ocean-based |
| <input type="checkbox"/> Maro Reef | | |
| <input type="checkbox"/> Laysan Island | <input type="checkbox"/> Land-based | <input type="checkbox"/> Ocean-based |
| <input type="checkbox"/> Lisianski Island, Neva Shoal | <input type="checkbox"/> Land-based | <input type="checkbox"/> Ocean-based |
| x Pearl and Hermes Atoll | <input type="checkbox"/> Land-based | x Ocean-based |
| x Midway Atoll | <input type="checkbox"/> Land-based | x Ocean-based |
| x Kure Atoll | <input type="checkbox"/> Land-based | x Ocean-based |
| <input type="checkbox"/> Other | | |

NOTE: Please note there is a fee schedule for people visiting Midway Atoll National Wildlife Refuge via vessel and aircraft.

Location Description:

island	site	depth(ft)	habitat	lat	long
ffs	r11b	67	lagoon	23 38.149	166 11.138
ffs	tc12	30	lagoon	23 38.323	166 10.802
ffs	tc21	25	forereef	23 50.822	166 19.630
ffs	r16	24	shelf	23 51.049	166 19.759
ffs	tc30	14	lagoon	23 50.988	166 17.840
ffs	cred 8	31	lagoon	23 51.034	166 19.826
ffs	rapture	80	patch	23 38.10	166 11.12
ffs	La Perouse	28	lagoon	23 46.77	166 15.695
ffs	cred 28	40	lagoon	23 50.562	166 19.2
PHR	jm10	3	backreef	27 50.072	175 45.210
PHR	tc31	19	backreef	27 46.532	175 58.401
PHR	tc32	21	backreef	27 46.351	175 56.370
PHR	tc26	5	backreef	27 57.468	175 48.125
PHR	r44	46	backreef	27 54.631	175 54.280
mid	tc1	3	backreef	28 16.148	177 23.181
mid	jm20	3	backreef	28 16.288	177 23.167
mid	r23-21	3	backreef	28 16.436	177 21.048
mid	r15	3	backreef	28 16.672	177 21.831
Kur	r36-10	15	backreef	28 25.198	178 22.345
Kur	tc17	12	backreef	28 25.912	178 22.003
Kur	tc13	3	backreef	28 27.147	178 18.915
Kur	tc14	3	backreef	28 27.209	178 19.716

5b. Check all applicable regulated activities proposed to be conducted in the Monument:

- x Removing, moving, taking, harvesting, possessing, injuring, disturbing, or damaging any living or nonliving monument resource

x Drilling into, dredging, or otherwise altering the submerged lands other than by anchoring a vessel; or constructing, placing, or abandoning any structure, material, or other matter on the submerged lands

x Anchoring a vessel

Deserting a vessel aground, at anchor, or adrift

x Discharging or depositing any material or matter into the monument

x Touching coral, living or dead

x Possessing fishing gear except when stowed and not available for immediate use during passage without interruption through the monument

Attracting any living monument resource

Sustenance fishing (Federal waters only, outside of Special Preservation Areas, Ecological Reserves and Special Management Areas)

Subsistence fishing (State waters only)

x Swimming, snorkeling, or closed or open circuit SCUBA diving within any Special Preservation Area or Midway Atoll Special Management Area

6. Purpose/Need/Scope *State purpose of proposed activities:*

Global climate change and human activities are placing coral reef ecosystems at risk. Coral reefs worldwide are now declining at an alarming rate. Mass bleaching events have increased dramatically since the 1980's and have usually been linked to El Nino or global warming-related increases in annual sea surface temperature (Brown 1997, Barber et al. 2001). The El Nino Southern Oscillation (ENSO) conditions during 1997 to 1998 resulted in worldwide bleaching from the Western Atlantic to the Great Barrier Reef. ENSO events have increased in frequency and duration in the past two decades (Barber et al. 2001, Walker 2001) and it has been predicted that the frequency and severity of coral bleaching will also continue to rise (Hoegh-Guldberg 1999).

Disease in coral reef ecosystems has received great attention, particularly in the western Atlantic where coral disease has been incriminated in the marked degradation of reef habitats. (Santavy and Peters 1997, Green and Bruckner 2000). Coral disease is reported to be responsible for the dramatic decline of Acroporids, one of the major frame-building corals in the Florida Keys, changing the structure and function of the coral reef ecosystem (Aronson & Precht 2001). Despite the major impact disease can have on reef systems, the etiology of most coral diseases remains unclear (Santavy and Peters 1997, Richardson 1998). The causative agents, mechanism of pathogenesis and link to environmental or anthropogenic stress are still largely unknown (Richardson 1998, Green & Bruckner 2000).

The reefs of the Northwestern Hawaiian Islands (NWHI) are considered to be relatively healthy but they are not immune to the conditions that have led to the decline of other reef systems. In September 2002 the first mass-bleaching event was recorded on the reefs of the NWHI. In the three northwestern most atolls of the Archipelago (Pearl & Hermes, Midway and Kure) over half of all sites had significant bleaching (Aeby et al. 2003, Kenyon et al., 2005). Ten coral disease states have now been described from the NWHI (Aeby 2006) and we have established permanent sites which allow us to determine both temporal and spatial changes in diseases through time and the ultimate affect of disease on the health of the ecosystem. We will measure changes in disease levels through time, rates of tissue loss from different diseases, patterns of disease transmission among colonies, rate of spread of disease and evaluate changes in coral cover and coral species composition. In addition, two diseases of concern have been identified, *Acropora* white syndrome and *Acropora* growth anomalies which we are targeting for focused studies.

Acropora white syndrome (AWS) is a disease which causes acute tissue loss in acroporids and has been reported from across the Indo-Pacific. *Acropora* white syndrome appeared on one reef in the northwestern Hawaiian Islands (NWHI) in 2003 (Aeby 2006) and has since spread. Our prior studies in 2005 and 2006 found this disease to be highly virulent having killed over 19 large table acroporids with numerous other colonies suffering massive tissue loss from the disease. The disease occurs predominantly at French Frigate Shoals (FFS) within the NWHI, which is the center of abundance and diversity of acroporids in Hawaii. We plan to continue to follow the dynamics of this disease but the important question is what is causing this disease. The etiology of AWS at FFS has not yet been investigated. From bacterial studies in 2006, we found infected coral to contain significantly higher numbers of culturable bacteria suggesting that culturable bacteria could be the cause of the infection as has been found elsewhere. Research by the Australian Institute of Marine Science (AIMS) in Australia, Palau and the Marshall Islands has found the disease to be caused by pathogens from the family vibrionaceae, which produce exocellular peptidases causing rapid photo inhibition, zooxanthelial

expulsion and tissue degradation ending in coral mortality (Bourne 2005, Sussman et al. 2005a, 2005b, 2006). In collaboration with scientists at the AIMS we propose to collect and preserve microbes from fragments of *Acropora cythera* for use in infection trials at secure PC2 facilities on Oahu.

Disease can affect coral communities directly through mortality of colonies (partial or whole) resulting in reduced coral cover (such as we found for AWS) or indirectly through sub-lethal events such as reduced growth, resilience or reproduction. From our 2006 study we discovered that *Acropora cythera* with growth anomalies suffer a significant reduction in reproductive output. We would now like to determine whether this disease also affects the growth of colonies.

Diseases in marine ecosystems are not only limited to corals. Fibropapillomatosis of green turtles has been known in Hawaii since the 1950s (Balaz 1991). More recently, high levels of infections with bacteria and protozoa have been seen in taape (*Lutjanus kasmira*) (Work et al. 2003). Taape were introduced into Hawaii in the 1950s (Randall 1987) and have spread all the way to Midway Atoll. Taape are closely associated with certain native fish such as goatfish (*Mulloidichthys* sp.) (Friedlander et al. 2002) and goatfish from the main Hawaiian Islands have been found to be infected with some of the same diseases as taape (Work et al. unpub. data). Given that taape were introduced into Hawaii, there is the concern that the recently documented diseases may have been introduced with them from the Marquesas. Taape are infected with a gut nematode that is thought to have been brought into the Hawaiian ecosystem with the introduction of the fish. This nematode infection has also been found in co-occurring native goatfish species. Taape were originally introduced into Oahu and have recruited out to other islands and up into the NWHI. The question now arises as to whether disease transmission has occurred from the main HI out to the NWHI.

From our 2006 study we found that taape from FFS had the nematode infection yet this disease was not found in fish from Midway. It appears that there is a lag in the time required for taape to establish in the NWHI as compared to the establishment of fish disease. The spread of both taape and its diseases up into the NWHI may be reflective of real time ecological linkages between islands within the Hawaiian archipelago. We have a rough timeline of the spread of taape from Oahu out to Midway and could correlate that with the eventual emergence of this disease at Midway. From studies in 2006, we also found that species of native goatfishes from FFS also have the nematode infection. We would like to also sample goatfishes from the other islands we are visiting to determine whether the pattern of disease is similar to that found in taape. From our 2005 and 2006 studies we found that the surgeonfish, *Ctenochaetus strigosus*, with a pigment discoloration had pathology consistent with cancerous lesions. We would like to conduct further studies of this disease.

It is important for management agencies to have a thorough understanding of the vulnerability of these reefs to disease and the first steps in managing disease are developing an understanding of the causes of disease and assessing its geographic extent. Management of disease in wildlife populations usually involves either reducing or removing the source of infection or reducing the spread of the disease. However, before appropriate management plans can be made the epizootiology of diseases must be understood. Our studies, past and proposed, are supplying critical information into disease dynamics in both coral and fish within the NWHI.

Objectives:

- 1) To re-survey permanent sites at FFS, Pearl and Hermes, Midway and Kure established in 2005 for the assessment of disease dynamics.
- 2) To survey additional *Acropora* rich sites within FFS
- 3) Determine the etiology of *AWS*
- 4) Determine whether *AWS* is infectious
- 5) Determine the affect of *Acropora* growth anomalies on the growth of table corals.
- 6) Examine *Lutjanus kasmira* and native goatfishes (*M. flavolineatus*, *Parupeneus multifasciatus*, *P. porphyreus*) for presence of disease.
- 7) Examine the surgeonfish, *Ctenochaetus strigosa*, affected by pigmentation disease.

7. As explained further in the instructions, please provide any information that you believe will assist the Co-Trustees in determining how your proposed activities are compatible with the conservation and management of the natural, historical and cultural resources of the Monument:

Justification:

Recently, outbreaks of novel diseases have occurred in the world's oceans. Mass mortality of sea fans occurred in reefs of the Caribbean and Florida Keys. The pathogen, *Aspergillus*, was thought to be a new species that originated from terrestrial sources (Smith et al., 1996, Rosenberg and Ben-Haim 2002). White pox, a lethal disease of *Acropora palmata*, was first documented on the reefs in 1996 and was found to be caused by a common fecal enterobacterium found in the human gut (Patterson et al. 2002). Current models of global climate change predict a significant increase in sea surface temperature (Kleypas et al. 1999, Walker 2001). Elevated temperatures have been shown to accelerate the growth rate and pathogenicity of pathogens (Porter et al., 2001) and so it has been predicted that coral disease will become even more common and widespread (Porter et al. 2001, Rosenberg and Ben-Haim 2002). Disease in the Indo-Pacific is on the rise (Willis et al. 2005, Jacobson 2006) and is damaging acroporid populations at FFS. Management of wildlife diseases requires an understanding of the disease dynamics on the reefs of the NWHI. This knowledge will be critical if we are to effectively address disease outbreaks and provide appropriate management recommendations to resource biologists.

8. Procedures:

Disease surveys: Re-survey of established sites at FFS, Pearl and Hermes (PHR), Midway (MID) and Kure (KUR) will follow established protocol. Two 25 m lines will be laid out along the permanent pins. A diver will then swim over the lines during which all corals within one half meter of either side of the transect lines will be identified to specie, counted, and assigned to a size class (0-5cm; 6-10cm; 11-20cm; 21-40cm; 41-80cm; 81-150cm; >150cm.). In the same manner, a second diver will swim over the lines and examine all corals for signs of bleaching or disease. Bleached colonies will be assigned a bleaching category: 0-no bleaching; 1- 10-30%; 2- 30-50%; 3-50-100%; 4- 100%; 5-mortality. For corals exhibiting disease, a general description of the condition will be recorded, the coral will be photographed and a specimen will be collected for histopathological examination. All enumerated bleached and diseased corals will also be assigned a size class consistent with the population counts. Individual colonies tagged in 2005 or 2006 will be relocated, remarked and photographed. Any new infected colonies along

the transect will be photographed and tagged. Any lost pins will be replaced and any loose pins re-glued.

Acropora growth studies

Colonies of *A. cytherea* with growth anomalies and a nearest neighbor of similar size will be measured (length and width of each tier), photographed (with a ruler) and tagged. They will be re-examined the following year (2008) to look for differences in growth between affected and control colonies. We will be tagging colonies located at our established permanent sites at FFS.

Etiology of AWS

Small fragments of infected coral and healthy fragments for control will be collected at depth and placed into individual bags. Samples will be held on ice and transported to the ship where they will be processed. To determine whether the disease is infectious, a subsample of the fragments will first be used for transmission studies. An infected fragment and a control fragment will be held together in a bucket of seawater for 24-48 hours. The buckets will have tight fitting lids with a small hole for an airline and will be placed in tubs of fresh water providing secondary containment. The tubs will be secured in a protected area on deck which was successfully done last year. After a maximum of 2 days, the coral fragments will be examined, photographed and processed. To process the samples, the fragments of coral will be crushed with a sterile mortar and pestle and mixed with 10ml of sterile seawater. The mixture will be placed in sterile tubes in 15% glycerol stock solution and frozen at -80°C. This procedure results in frozen microflora which will be re-cultured back on Oahu to conduct infection trials at a secure PC2 facility. Any seawater or equipment exposed to infected coral will be sterilized with 10% bleach solution which is a standard sterilization technique used in pathogen studies. To ensure that the transmission study is completed before leaving FFS, the study will be initiated immediately upon arrival at FFS. In addition, the chief scientist will be notified of the start and end of all trials so that can be taken into account if cruise schedules need to be adjusted.

Environmental screening for target microorganisms and specific virulence genes

Ideally, identification of disease would be accomplished through non-invasive techniques. As the etiology of diseases become known then DNA can be collected from the field using swabs or sterile syringes and screened for target microorganisms and specific virulence genes. To investigate the potential use of environmental screening, we would like to collect seawater and sediment adjacent to infected colonies and swab or syringe the mucus from infected and healthy colonies. Samples will be collected in the field, held on ice and transported to the ship where they will be frozen for later molecular analysis. At HIMB and/or AIMS, DNA will be extracted, amplified and microorganisms identified. Once the etiology of AWS at FFS is determined we will be able to determine the efficiency of this type of environmental screening. This work is part of an ongoing study at the AIMS and will be completed in collaboration with the scientists at the AIMS.

Fish disease studies

Target species of fish will be collected by spear, placed on ice and transported to the ship for examination. Fish will be weighed and measured (standard and fork length), examined systematically externally and internally, and gross lesions documented. For histopathology,

sections of skeletal muscle, skin, spleen, liver, cranial and caudal kidneys, swim bladder, brain, heart, gill, and gonad, small intestines, and stomach will be excised and fixed in 10% neutral buffered formalin. Tissues will be sectioned, dehydrated in alcohol series, embedded in paraffin, sectioned at 5 µm, placed on microscope slides, stained with hematoxylin and eosin, and examined using a light microscope. Special stains will be used as appropriate to identify fungi, bacteria, or protozoa. Histopathology will allow us to characterize microscopic morphology of disease, will provide systematic evaluation of cellular changes that occur in disease, and will afford the opportunity to detect microorganisms and the host response to these organisms.

For electron microscopy, tissue will be fixed in Trump's fixative (McDowell & Trump, 1976), rinsed in 0.1M Sorenson's phosphate buffer, and post fixed in 2% osmium tetroxide. Tissues will be embedded in epoxy, cut into 1-micron thick toluidine blue stained sections, ultra thin sections stained with uranyl acetate, post stained with lead citrate and examined with a Zeiss EM 109 electron microscope. Electron microscopy examines tissues at the sub-cellular level and allows one to characterize disease based on changes in cell organelles and to examine and identify, structurally, the interaction between foreign organisms and coral tissue. Very small organisms, such as viruses, can also be visualized using electron microscopy. For gut parasites, the entire intestinal tract will be removed and frozen for later analysis at HIMB. Targeted fish species include *Lutjanus kasmira*, *Ctenochaetus strigosus*, *Mulloidichthys vanicolensis*, *M. flavolineatus*, *Parupeneus multifasciatus*, *P. pleurostigma*, and *M. pflugeri*. Since processing of fish requires time, we are requesting permission to continue processing samples even if the ship travels out of Monument waters (such as to dump grey water).

Specimen collection. Fish will be collected by spear. Coral fragments will be collected by hammer and chisel or bone cutters.

Section C: Logistics

9. Other permits (list and attach documentation of all other related Federal or State permits):

prior permits: FWS permit #'s: 12521-04020, 12521-05017, 12521-05056, 12521-06028;
NWHICRER permit #'s: 2005-001,2005-013; DAR permit #'s: 2004-10, 2005-11, 2006-06,
06R008

9a. For each of the permits listed, please identify any permit violations or any permit that was suspended, amended, modified or revoked for cause. Please explain the circumstances surrounding the violation or permit suspension, amendment, modification or revocation.

10. Funding sources (Please attach copies of your budget, specific to proposed activities under this permit and include funding sources. Please see instructions for more information):

The project will be funded by the NWHICRER-HIMB partnership NMSPO MOU 2006-008/66882.

11. Time frame:

Activity start:2002

Activity completion: to be determined

Dates actively inside the Monument:

NWHI Monument
Permit Application
Page 10 of 25
From: Aug 3, 2007
To: aug 20, 2007

Please describe any limiting factors in declaring specific dates of the proposed activity at the time of application: These dates may change dependent upon weather or the ship;s schedule.

Personnel schedule in the Monument: All personnel will be onboard the Hi'ialakai which will be in the following locations:

FFS-8/4-8/7/07
PHR-8/10-8/14/07
MID-8/15-8/16/07
KUR-8/18-8/20/07

12. Please indicate (with attached documentation) what insurance policies, bonding coverage, and/or financial resources are in place to pay for or reimburse the Monument trustees for the necessary search and rescue, evacuation, and/or removal of any or all persons covered by the permit from the Monument: NOAA Ship HI'IALAKAI is a U.S. Government-owned and -operated research vessel and is self-insured by the U.S. Government.

13. Please check the appropriate box to indicate how personnel will enter the Monument:

Vessel
 Aircraft

Provide Vessel and Aircraft information:
NOAA ship Hi'ialakai- permit # pending

14. What certifications/inspections do you have scheduled for your vessel?

Rodent free, Date:
 Tender vessel, Date:
 Ballast water, Date:
 Gear/equipment, Date:
 Hull inspection, Date:

15. Vessel information (NOTE: if you are traveling aboard a National Oceanic and Atmospheric Administration vessel, skip this question):

Vessel name:

Vessel owner:
Captain's name:
IMO#:
Vessel ID#:
Flag:
Vessel type:
Call sign:
Embarkation port:
Last port vessel will have been at prior to this embarkation:
Length:
Gross tonnage:
Total ballast water capacity volume (m3):
Total number of ballast water tanks on ship:
Total fuel capacity:
Total number of fuel tanks on ship:
Marine Sanitation Device:
Type :

How will you comply with the 'No Discharge' regulations stipulated in Presidential Proclamation 8031? Describe in detail. If applicable, please attach schematics of the vessel's discharge and treatment systems:

Other fuel/hazardous materials to be carried on board and amounts:

Please provide proof of a National Oceanic and Atmospheric Administration (NOAA) Office of Law Enforcement-approved Vessel Monitoring System (VMS). Please provide the name and contact information of the contractor responsible for installing the VMS system. Please also describe unit name and type:

VMS Email:
Inmarsat ID#:

16. Tender information:

On what workboats (tenders) will personnel, gear and materials be transported within the Monument? Please list the number of tenders/skiffs aboard and specific types of motors: Personnel, gear and materials may be transported within the Monument by the ship or any of the 5 ship's small boats listed above or by the program-provided small boat listed below.

Ship's own tenders - 1 each 10 m AMBAR Marine jet boat with Yanmar 370-hp,
Diesel inboard engine
1 each 8 m AMBAR Marine jet boat with Yanmar 315-hp,
Diesel inboard engine

2 each 17.5 ft Zodiac inflatable boats, each with one Honda
50-hp, 4-stroke, outboard gasoline engine

1 each 19 ft AMBAR Marine rescue boat with Honda 115-
hp, 4-stroke, outboard gasoline engine

Program-provided tenders – 19' Boston Whaler with 135 hp Honda four-stroke outboard

Section D: Additional Information for Land Based Operations

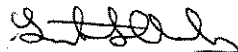
17. Proposed movement of personnel, gear, materials, and, if applicable, samples:

18. Room and board requirements on island:

19. Work space needs:

With knowledge of the penalties for false or incomplete statements, as provided by 18 U.S.C. 1001, and for perjury, as provided by 18 U.S.C. 1621, I hereby certify to the best of my abilities under penalty of perjury of that the information I have provided on this application form is true and correct.

Signature



Date 2/1/07

PLEASE SEND ONE SIGNED APPLICATION VIA MAIL TO THE MONUMENT OFFICE BELOW:

NWHI Marine National Monument Permit Coordinator
6600 Kalaniana'ole Hwy. # 300
Honolulu, HI 96825
FAX: (808) 397-2662

DID YOU INCLUDE THESE?

- Applicant CV/Resume/Biography
- Electronic and Hard Copy of Application with Signature
- Map(s) or GPS point(s) of Project Location(s), if applicable
- Funding Proposal(s)
- Funding and Award Documentation, if already received
- Documentation of Insurance, if already received
- Documentation of Inspections
- Documentation of all required Federal and State Permits or applications for permits
- Statement of information you wish to be kept confidential

Appendix A: Research OR Conservation and Management Application

NOTE: If land or marine archeological activities are involved, please contact the Monument Permit Coordinator at the address on the general application form before proceeding, as a customized application will be needed. For more information, please contact the Monument office on the first page of this application.

1a. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name: table coral

Scientific name: *Acropora cytherea*

& size of specimens: 40 fragments 2-5 cm each. 20 from AWS colonies and 20 from control colonies.

Collection location: FFS

Whole Organism Partial Organism

1b. What will be done with the specimens after the project has ended? The frozen samples will be transported to Oahu for molecular analysis and infection trials.

1c. Will the organisms be kept alive after collection? Yes No

• Specific site/location: Hi'ialakai. Half of the samples will be processed in the wetlab shortly after arrival on the ship and the other half will be maintained in closed buckets in secondary containment tubs secured on the ships deck for 1-2 days before processing.

• Is it an open or closed system? Open Closed

• Is there an outfall? Yes No

• Will these organisms be housed with other organisms? If so, what are the other organisms? no

• Will organisms be released? no

2. If applicable, how will the collected samples or specimens be transported out of the Monument? Samples will be transported on ice on small boats to the NOAA ship which may or may not be within Monument waters. Frozen samples will be transported back to Honolulu via the NOAA ship.

3. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research: Samples will be shared with Rob Toonen for molecular studies.

1a1. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name: reef coral

Scientific name: *species will vary*

& size of specimens: We anticipate a maximum of 30 samples (all islands combined) to be collected if new diseases are encountered. Sample size would be 2-5 cm each. 2 samples would be taken per colony (one from the diseased region and one from the healthy region).

Collection location: FFS, PHR, MID, KUR

Whole Organism Partial Organism

1b. What will be done with the specimens after the project has ended? The samples will be fixed in Z-fix and transported to Oahu for histopathological analysis.

1c. Will the organisms be kept alive after collection? Yes No

• Specific site/location: Hi'ialakai. If it is from a known disease the sample will be fixed onboard the small dive boats. However, sometimes our investigations require that corals be examined under the microscope before fixing. In those cases, corals will be transported live in buckets of sea water to the Hi'ialakai. After examination the sample will be placed in Z-fix.

• Is it an open or closed system? Open Closed

• Is there an outfall? Yes No

• Will these organisms be housed with other organisms? If so, what are the other organisms? no

• Will organisms be released? no

2. If applicable, how will the collected samples or specimens be transported out of the Monument? Samples will be transported in buckets of seawater on small boats to the NOAA ship which may or may not be within Monument waters. Fixed samples will be transported back to Honolulu via the NOAA ship.

3. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research: Samples will be shared with Rob Toonen for molecular analysis.

1a2. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name: reef fish

Scientific name: Targeted fish species include *Lutjanus kasmira*, *Ctenochaetus strigosus*, *Mulloidichthys vanicolensis*, *M. flavolineatus*, *Parupeneus multifasciatus*, *P. pleurostigma*, and *M. pflugeri*

& size of specimens: We will collect a maximum of 20 fish per specie per island for *Lutjanus kasmira* and the five goatfish species. For *Ctenochaetus strigosus* we are requesting an additional 5 healthy fish as controls for a total of 25 fish. *Lutjanus kasmira* are not yet known from Kure but if they have spread that far we would like to examine them for disease to get a better idea of the time frame for disease spread within the Hawaiian archipelago. *Ctenochaetus strigosus* with pigmentation disease has only been reported from FFS, however, if encountered on other islands we would like to determine if the histopathology of the disease is the same as at FFS.

Collection location: FFS, PHR, MID, Kur

Whole Organism Partial Organism

1b. What will be done with the specimens after the project has ended? The frozen and fixed samples will be transported to Oahu for parasitological and histopathological analysis.

1c. Will the organisms be kept alive after collection? Yes No

• Specific site/location:

• Is it an open or closed system? Open Closed

• Is there an outfall? Yes No

• Will these organisms be housed with other organisms? If so, what are the other organisms?

• Will organisms be released?

2. If applicable, how will the collected samples or specimens be transported out of the Monument? Samples will be transported on ice on small boats to the Hi'ialakai which may or may not be within Monument waters. Frozen and fixed samples will be transported back to Honolulu via the Hi'ialakai.

3. Describe collaborative activities to share samples, reduce duplicative sampling, or duplicative research: Fish samples will be collected by and shared with Brian Bowen's group and so they will also be used for both molecular and life history studies.

4a. Gear and materials:

dive gear

coral collection gear (bone cutters, hammer, chisel, ziplock and whirlpak bags, bag to carry gear)

coral processing gear (plastic jars, z-fix)
stereo microscope
fish dissecting gear (scissors, scalpels, forceps, scale, rulers, plastic jars, formalin)
cameras and underwater housing
sludge hammer, steel pins and underwater glue
field equipment (tape measures, floats, clipboards, underwater paper, cow ear tags, cable ties)
hand held GPS
computer
IPOD
5 gal buckets with lids
tubs for secondary containment
aquarium pumps with tubing
thermometers
duct tape, bungee cords and line to secure tubs and buckets
mortar and pestles
nalgene bottles
graduated cylanders
beakers
sterile tubes
filtering unit
ice chests
rubbermaid containers
Miscellaneous office supplies (books, tablets, pencils, pens, markers, scissors, stapler, 3-hole punch, etc.)
Personal gear (clothing, personal hygiene items, diet coke, snacks, sunglasses, etc)

4b. Please list all Hazardous Materials you propose to take to and use within the Monument:

Clorox
Z-fix
Ethanol
Gluteraldehyde
Formaldehyde

5. Fixed installations and instrumentation: Repair or replacement of steel pins at permanent monitoring sites.

6. Provide a time line for sample analysis, data analysis, write-up and publication of information: Fall 2007: histology and parasitology processing. Spring 2008: data analysis and report writing

7. List all publications directly related to the proposed project:

- Aeby, G.S. 2006. Baseline levels of coral disease in the Northwestern Hawaiian Islands. *Atoll Research Bulletin* 543:471-488.
- Aeby, G.S. 2006. Outbreak of coral disease in the Northwestern Hawaiian Islands. *Coral Reefs* 24(3):481.
- Aeby, G. S., Kenyon, J., Maragos, J. and Potts, D. 2003. First record of mass coral bleaching in the Northwestern Hawaiian Islands. *Coral Reefs* 22:256.
- Aronson, R. B. and W. F. Precht. 2001. White-band disease and the changing face of Caribbean coral reefs. *Hydrobiologia*. 460: 25-38.
- Barber, R., A. Hiltling, and M. Hayes. 2001. The changing health of coral reefs. *Human and Ecological Risk Assessment*: 7(5):1255-1270.
- Bourne, D.G. (2005) Microbial assessment of a disease outbreak on coral from Magnetic Island (Great Barrier Reef, Australia). *Coral Reefs* 24:304-312.
- Brown, B. 1997. Coral bleaching: causes and consequences. *Coral Reefs* 16:S129-S138.
- Friedlander AM, Parrish JD, DeFelice RC (2002) Ecology of the introduced snapper *Lutjanus kasmira* (Forsskal) in the reef fish assemblage of a Hawaiian Bay. *J Fish Biol* 60:28-48
- Green, E. and Bruckner, A. 2000. The significance of coral disease epizootiology for coral reef conservation. *Biological Conservation*. 96: 347-361.
- Harvell, C., Kim, K., Burkholder, J., Colwell, R., Epstein, P., Grimes, D., Hofmann, E., Lipp, E., Osterhaus, A., Overstreet, R., Porter, J., Smith, G., & Vasta, G. 1999. Emerging marine diseases—Climate links and anthropogenic factors. *Science* 285:1505-1510.
- Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Marine Freshwater Research* 50:839-866.
- Jacobson, D. 2006. Fine Scale Temporal and Spatial Dynamics of a Marshall Islands Coral Disease Outbreak: Evidence for Temperature Forcing. (abstract) Ocean Sciences meeting.
- Kenyon, J.C., Aeby, G., Brainard, R., Chojnacki, J., Dunlap, M. and C. Wilkinson
In press. Mass coral bleaching on high-latitude reefs in the Hawaiian Archipelago. *Proceedings of the 10th Int. Coral Reef Symposium, Okinawa.*
- Kleypas, J., Buddemeier, R., Archer, D., Gattuso, J., Langdon, C, and Opdyke, B. 1999. Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. *Science* 284:118-120.
- Patterson, K., Porter, J., Ritchie, K., Polson, S., Mueller, E., Peters, E., Santavy, D., and Smith G. 2002. The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. *Proceedings of the New York Academy of Sciences*. 99: 8725-8730.
- Peters, E. 1997. Diseases of coral reef organisms. In: Birkeland, C. (Ed.). *Life and Death of Coral Reefs*. Chapman & Hall, London, pp.114-136.
- Porter, J., P. Dustan, W. Jaap, K. Patterson, V. Kosmynin, O. Meier, M. Patterson, and M. Parsons. 2001. Patterns of spread of coral disease in the Florida Keys. *Hydrobiology* 159: 1-24.
- Randall JE (1987) Introduction of marine fishes to the Hawaiian Islands. *Bull Mar Sci* 41:490-502
- Richardson, L. 1998. Coral diseases: what is really known? *Trends in Ecol. Evol.* 13 (11):438-443.

- Rosenberg, E., and Ben-Haim, Y. 2002. Microbial diseases of corals and global warming. *Environ Microbio* 4(6):318-326.
- Santavy, D., Peters, E. 1997. Microbial pests: Coral disease in the Western Atlantic. *Proc 8th Int Coral Reef Sym* 1:607-612.
- Santavy, D., Mueller, E., Peters, E., MacLaughlin, L., Porter, J., Patterson, K. & Campbell, J. 2001. Quantitative assessment of coral diseases in the Florida Keys: strategy and methodology. *Hydrobiologia*. 460: 39-52.
- Smith, G., et al. 1996. Caribbean sea fan mortalities. *Nature*. 383: 487.
- Sussman, M, Willis, B, Bourne, D. (2005a) Investigation of a causative agent for Degenerating Bleaching Disease (DBD) affecting tabular *Montipora* sp. corals on the GBR. Abstract. Australian Society for Microbiology, Canberra, Australia
- Sussman M, Bourne, DG, Page C, Jacobson, D, Willis, B. (2005b) Isolation and identification of the causative agent for a white syndrome coral epizootic in the Marshall Islands. Abstract. Estuarine Research Federation, Norfolk, Virginia
- Sussman M, Willis, B, Bourne, D, Raymundo, L, Safavi, H, Victor, S, Morris, A, Doyle, J, Harvell, D. (2006) The ecology of virulence: applying new screening methods for the identification of a causative agent for a white syndrome coral epizootic in Palau. Abstract. Ocean Sciences Meeting, Honolulu, HI.
- Walker, H. 2001. Understanding and managing the risks to health and environment from global atmospheric change: A synthesis. *Human and Ecol Risk Assessment* 7(5):1195-1209.
- Work T, Rameyer RA, Takata G, Kent M. 2003. Protozoal and epitheliocystis-like infections in the introduced blueline snapper *Lutjanus kasmira* in Hawaii. *Diseases of Aquatic Organisms* 37:59-66.

DID YOU INCLUDE THESE?

X Material Safety Data Sheets for Hazardous Materials

Appendix B: Education Application

1. Are you collaborating with others in any way to reduce duplicative activities in the Monument or elsewhere?

2. Gear and materials:

3. Fixed installations and instrumentation:

4. Is your proposed activity based on a State Department of Education Standards Based Curriculum? If so, please describe:

5. What materials, products or deliverables will be developed as a result of your proposed activity? Provide a time line for write-up and publication of information or production of educational materials:

6. List all publications/references directly related to the proposed project:

7a. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name:

Scientific name:

& size of specimens:

Collection location:

Whole Organism Partial Organism

7b. What will be done with the specimens after the project has ended?

7c. Will the organisms be kept alive after collection? Yes No

• Specific site/location:

• Is it an open or closed system? Open Closed

• Is there an outfall? Yes No

• Will these organisms be housed with other organisms? If so, what are the other organisms?

• Will organisms be released?

8. If applicable, how will the collected samples be transported out of the Monument?

Appendix C: Native Hawaiian Practices Application

1. Please state how the purpose and intent of the activity are appropriate and deemed necessary by traditional standards in the Native Hawaiian culture (pono), and demonstrate an understanding of, and background in, the traditional practice, and its associated values and protocols:

2. Please state how the activity benefits the resources of the Northwestern Hawaiian Islands and the Native Hawaiian community:

3. Please state how the activity supports or advances the perpetuation of traditional knowledge and ancestral connections of Native Hawaiians to the Northwestern Hawaiian Islands:

4. Will you be collecting any Monument resource? Yes No
If so, please provide the following information:

4a. Collection of specimens - collecting activities (would apply to any activity): organisms or objects (List of species, if applicable, attach additional sheets if necessary):

Common name and/or Scientific name:

& size of specimens:

Collection location:

Whole Organism Partial Organism

4b. What will be done with the specimens after the Native Hawaiian cultural practice is complete?

4c. Will organisms be kept alive after collection? Yes No

• Specific site/location:

• Is it an open or closed system? Open Closed

• Is there an outfall? Yes No

• Will these organisms be housed with other organisms? If so, what are the other organisms?

• Will organisms be released?

NOTE: Any Monument resource harvested from the Monument for the purpose of Native Hawaiian practices will be consumed in the Monument.

5. Are you collaborating with others in any way to reduce duplicative activities in the Monument or elsewhere?

6. Gear and materials:

7. Will you erect any Native Hawaiian cultural structures or leave any offerings in the Monument? Yes No

If so, please describe:

8. Will you produce any publications, educational materials or other deliverables?
 Yes No

Provide a time line for write-up and publication of information or production of materials:

Appendix D: Recreation Application

For Activities in the Midway Atoll Special Management Area Only

- 1. Please explain how the activity is for the purpose of recreation as defined: An activity conducted for personal enjoyment that does not result in the extraction of Monument resources and that does not involve a fee-for-service transaction:**
- 2. Other Associated Monument Permits:**
- 3. Gear and materials:**
- 4. Fixed installations and instrumentation:**

Appendix E: Special Ocean Use Application

NOTE: If this is a first time Special Ocean Use activity, it will be subject to a pilot project.

- 1. Please provide proof of general liability insurance, or indicate that you will be posting an equivalent bond against claims arising out of activities conducted under the permit:**
- 2. Are you collaborating with others in any way to reduce duplicative activities in the Monument or elsewhere?**
- 3. Gear and materials:**
- 4. Fixed installations and instrumentation:**
- 5. List all publications directly related to the proposed project:**

For projects occurring with the Midway Atoll Special Management Area answer the following questions:

- 6. Please explain how your activity has been found compatible with the purposes for which the Midway Atoll National Wildlife Refuge was designated?**
- 7. Please explain how your activity meets the requirement of furthering conservation and management of the Monument:**

For projects occurring outside of the Midway Atoll Special Management Area answer the following questions:

- 8. Please explain how the proposed activity will directly benefit the conservation and management of the Monument:**
- 9. Please explain how the purpose of the proposed activity is for research and education related to resources or qualities of the Monument:**

NOTE: SPECIAL OCEAN USE PERMITS OUTSIDE THE MIDWAY ATOLL SPECIAL MANAGEMENT AREA DO NOT ALLOW THE USE OF A COMMERCIAL PASSENGER VESSEL. A commercial passenger vessel is defined by the monument regulations as a vessel that carries individuals who have paid for such carriage.