

Appendix 1: Gates Permit 2008

Sampling Strategy and Collection Request for 2008

The sample taken from each coral is a very small biopsy (<1cm). The impact of this sampling to is significantly less damaging to the coral than marine organisms that feed on coral in the area (e.g. parrot fish). The sampling impacts are thus extremely minimal and pose no significant threat to the survival of the colony sampled. For other marine invertebrate hosts, a clipping of one of the tentacles will be taken which is non-lethal and has almost no effect to the organism. Analysis of the microscopic foraminiferan hosts involves taking the entire organism (which is approximately 1- 5 mm in size). The removal of the few specimens for this analysis will have no effect on the populations of these microorganisms, which are very abundant (>1,000,000 per m²).

Samples that overlap between collections outlined below represent a single sample that will count towards both sample sets. As the itinerary for the research cruise has not been scheduled, a sampling strategy that provides the freedom to sample at all the atolls is included. Realistically, only a few of the atolls will be visited and so the number of samples collected will be much less than that outlined below.

1. Collections to identify and define the types of symbiotic dinoflagellates harbored by healthy and diseased corals

Corals belonging to the genera *Acropora* and *Pocillopora* have been chosen for this study because both are sensitive to environmental disturbance and bleach more readily than other species. In addition, data resulting from the 2005 and 2006 collections revealed that diseased *Acropora cytherea* harbor a rare and very different type of symbiont than their healthy counterparts at French Frigate Shoals (Stat et al in prep). *Porites lobata* and *Montipora capitata* have also been chosen for this study because they are common and are widely distributed within the Hawaiian archipelago and throughout the Pacific, they exhibit differences in their susceptibility to bleaching and disease, and both species are being developed as models for work focusing on functional aspects of the symbiosis being conducted at HIMB. In addition, ongoing sampling for this objective is important for understanding the changes in the symbiont community that occur over time as a result of increases in coral bleaching and disease.

We are requesting a permit to cover the collection of thirty corals (a statistically relevant number) representing each species and health state, for all ten atolls. In reality, the number of samples collected at each location in Papahānaumokuākea will reflect the incidence of disease and/or bleaching, and our past experiences suggest that individuals representing the compromised health states are rarely encountered. For example, on the September 2005 expedition, coral disease was limited to a single host species, *A. cytherea*, found at one reef location, French Frigate Shoals. As such, we have designed a sampling strategy that provides the researchers the flexibility to take advantage of chance encounters with diseased and/or bleached individuals of the target species at each site visited but that translates as a substantial overestimation of the actual number of corals that will be collected. In addition, the cruise schedule of the Hi'ialakai has not been determined and therefore we are listing all ten atolls

within the monument for this objective, however it is very unlikely that we will visit all of the atolls.

Coral Species (n = 10 for healthy, n = 20 for unhealthy at each atoll)

Nihoa

Coral Species	Common Name	Sample Number
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

Necker

Coral Species	Common Name	Sample Number
<i>Acropora cytherea</i>	Table Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

French Frigate Shoals

Coral Species	Common Name	Sample Number
<i>Acropora cytherea</i>	Table Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

Gardner

Coral Species	Common Name	Sample Number
<i>Acropora cytherea</i>	Table Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

Maro

Coral Species	Common Name	Sample Number
<i>Acropora cytherea</i>	Table Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

Laysan

Coral Species	Common Name	Sample Number
<i>Acropora cytherea</i>	Table Coral	30
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

Lisianski

Coral Species	Common Name	Sample Number
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

Pearl and Hermes

Coral Species	Common Name	Sample Number
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

Midway

Coral Species	Common Name	Sample Number
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

Kure

Coral Species	Common Name	Sample Number
<i>Pocillopora damicornis</i>	Lace Coral	30
<i>Pocillopora meandrina</i>	Cauliflower Coral	30
<i>Porites lobata</i>	Lobe Coral	30
<i>Montipora capitata</i>	Rice Coral	30

Total number request of coral samples for disease study: 1230 biopsies

Estimate of actual collection: < 250 coral biopsies

Note that the majority of samples (67%) will be collected from diseased and bleached coral and therefore will not affect healthy coral colonies. Corals species sampled collection 1 (described above) and collection 2 (described below) that overlap represent a single sample.

2. Collections to define the diversity and distribution of symbiotic dinoflagellates across Papahānaumokuākea

This study focuses on the types of symbiotic dinoflagellates found in a diversity of corals, other marine invertebrates, foraminifera, sea water and sediment, sampled at locations that cross the archipelago (see below) and is a continuation of work initiated in 2005. To date we have a portion of the total coral sample request collected from French Frigate Shoals, Nihoa, Pearl and Hermes, Lisianski and Gardner. To fill in the geographic and host gaps in this dataset we are requesting a permit to collect samples from additional atolls, additional coral species, soft coral, zoanthid, jellyfish, and foraminifera hosts (which are a source pool of coral endosymbionts), the sediment (source of free-living coral endosymbionts) and water (source of free-living coral endosymbionts).

The sample number for a host species that has been reached on previous cruises will not be re-sampled in upcoming cruises, eg. *Pavona varians* will not be sampled from French Frigate Shoals or Pearl and Hermes as a total of 5 colonies at each of these atolls has been collected on previous cruises.

1. Marine Invertebrates:

Total is 5 colonies per species per atoll (27 species X 5 colonies X 10 atolls)

Coral Species	Common Name
<i>Acropora humilis</i> *	Finger Staghorn Coral
<i>Acropora cytherea</i>	Table Coral
<i>Acropora nasuta</i>	Branching staghorn coral
<i>Pocillopora damicornis</i>	Lace Coral
<i>Pocillopora meandrina</i>	Cauliflower Coral
<i>Pocillopora eydouxi</i>	Antler Coral
<i>Pocillopora ligulata</i> *	Cauliflower Coral
<i>Porites brighami</i>	Brigham's Coral
<i>Porites lichen</i>	Lichen Coral
<i>Porites lobata</i>	Lobe Coral
<i>Porites duerdeni</i> *	Thick Finger Coral
<i>Porites solida</i> *	Solid Coral
<i>Montipora capitata</i>	Rice Coral
<i>Montipora patula</i>	Sandpaper Rice Coral
<i>Montipora flabellata</i> *	Blue Rice Coral
<i>Leptastrea bewickensis</i>	Bewick Coral
<i>Pavona varians</i>	Corrugated Coral
<i>Pavona duerdeni</i> *	Porkchop Coral
<i>Fungia scutaria</i>	Oval Mushroom Coral
<i>Gardineroseris planulata</i> *	Honeycomb coral
<i>Cyphastrea ocellina</i> *	Ocellated Coral
<i>Leptoseris mycetoseroides</i> *	Ridge Coral
<i>Psammocora niertrazi</i> *	Nierstrasz' Coral

Other Invertebrate hosts

<i>Aiptasia pulchella</i> *	Glass, Tube, Rock Anemone
<i>Palythoa caesia</i> *	Pillow Zoanthid
<i>Sarcothelia edmondsoni</i> *	Blue Soft Coral
<i>Cassiopea sp.</i> *	Upside-Down Jellyfish

Total number request of marine invertebrate samples for diversity study: 1350

Number collected to date: 177

Number remaining to collect: 1173

Estimate of actual final collection: 600 (This is due to most of the species listed above found at only some of the atolls)

2. Foraminifera

Total is 20 forams per species per atoll (3 species X 20 colonies X 10 atolls)

*Amphisorus hemprichii**

*Marginopora kudakajimaensis**

*Sorites sp**

Total number request of foram samples for diversity study: 600

Number collected to date: 0

Estimate of actual final collection: 300

3. Water and sediment

A total request of 20 5L water samples and 20 1 ml sediment samples per atoll will be collected during this permit activity to monitor free-living coral endosymbiont communities.

Total number request of water and sediment samples: 400 ((20 water + 20 sediment) X 10 atolls)

Estimate of actual final collection: 200

* Denotes additional new host to sample