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## **CRUISE REPORT<sup>1</sup>**

**VESSEL:** NOAA Ship *Hi'ialakai*, Cruise HA-13-05

**CRUISE PERIOD:** 3–19 September 2013

**AREA OF OPERATION:** Northwestern Hawaiian Islands, specifically French Frigate Shoals, Pearl and Hermes Atoll, and Lisianski Island, and O`ahu Island of the main Hawaiian Islands

**TYPE OF OPERATION:** Personnel from the Coral Reef Ecosystem Division (CRED) of the NOAA Pacific Islands Fisheries Science Center, University of Hawai`i at Mānoa (UHM), Scripps Oceanographic Institution (SIO), and San Diego State University (SDSU) conducted interdisciplinary surveys of benthic community composition and oceanographic parameters related to climate change in coastal waters across the Northwestern Hawaiian Islands and the west side of O`ahu Island as part of the National Coral Reef Monitoring Plan (NCRMP). All activities described in this report were covered by the following permits: Papahānaumokuākea Marine National Monument Permit No. 2013-001 (effective date: 03 September 2013, expiration date: 19 September 2013); Hawai`i Department of Land and Natural Resources, Division of Aquatic Resources Special Activity Permit No. 2014-6 (effective date: 19 July 2013, expiration date: 19 July 2014); U. S. Army Corps of Engineers Permit: USACE POH-2008-83 (OMB APPROVAL NO. 0710-0003) 30 April 2009.

## **ITINERARY:**

Note: Daily field operations included the establishment of survey sites for the climate study part of NCRMP around each island or atoll in the cardinal directions (N, S, E, W) and the exchange of subsurface temperature recorders (STRs) deployed on the benthos to

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<sup>1</sup> PIFSC Cruise Report - CR-15-004  
Issued March 31, 2015

continue collection of temperature data for a time series that in many locations has high-resolution data for up to a decade. Because this cruise was dedicated to climate research, a single NCRMP survey site included methods for collecting biological and chemical data related to monitoring the thermal structure of the water column along the forereef and monitoring the temporal and spatial variability in carbonate chemistry at the reef, while evaluating the associated biological responses to the effects of increasing water temperatures and ocean acidification. Every NCRMP site typically is established on the forereef at a depth of 15 m. At this depth at each site, 3 autonomous reef monitoring structures (ARMS), 5 calcification accretion units (CAUs), 5 bioerosion monitoring units (BMUs), and 1 STR are deployed and 3 discrete water samples are collected (and later analyzed for dissolved inorganic carbon [DIC], total alkalinity [TA], and salinity) in concert with a conductivity-temperature-depth (CTD) cast. Additionally, reef composition at the NCRMP site is studied through analysis of photographs taken during line-point-intercept surveys and rugosity measurements. Incorporated through the NCRMP site is a transect where additional STRs are deployed perpendicularly from shore at depths of 1, 5, and 25 m. From the NOAA Ship *Hi'ialakai* CTD casts were conducted, when the ship schedule and weather allowed, to a 300-m depth at 6–10 stations around each island on the same N, S, E, W directions in which the NCRMP sites were established. Additionally, a scientist from SDSU collected water samples for microbial studies (bacterial and viral meta-genome analyses and discrete dissolved organic carbon [DOC] analysis), and SIO scientists captured photographic records of the benthos over an area of 100 m<sup>2</sup> at NCRMP survey sites when conditions allowed. Unless otherwise specified in the following daily summaries, these activities occurred during each operational day.

- 3 September    Start of cruise, embarked all scientific personnel. Routine departure-day activities, including dive safety and operational briefings, dive gear checks, safety drills, etc. Transit to French Frigate Shoals.
  
- 4 September    Transit to French Frigate Shoals.
  
- 5 September    Arrived French Frigate Shoals ~ 1000, commenced field operations. Established 1 NCRMP site and retrieved existing ARMS, CAUs, and STRs. Conducted 7 nighttime shipboard CTD hydrocasts to 300-m depth as part of an offshore transect.
  
- 6 September    Continued operations at French Frigate Shoals. Established 2 NCRMP sites and retrieved existing ARMS, CAUs, STRs, and 1 ecological acoustic recorder (EAR). Conducted 5 nighttime shipboard CTD hydrocasts to 300 m depth as part of an offshore transect.
  
- 7 September    Continued operations at French Frigate Shoals. Established 1 NCRMP site and retrieved existing ARMS, CAUs, and STRs.
  
- 8 September    Transit to Pearl and Hermes Atoll. Processed/photographed/sorted the ARMS which had been recovered from French Frigate Shoals.

- 9 September Transit to Pearl and Hermes Atoll. Processed/photographed/sorted the ARMS which had been recovered from French Frigate Shoals.
- 10 September Arrived Pearl and Hermes Atoll ~ 0600, commenced field operations. Established 2 NCRMP sites and retrieved existing ARMS, CAUs, STRs, and 1 EAR. Discovered a very large derelict fishing net (estimated to be 625 m<sup>3</sup> in volume) that was partially snagged on the NW forereef. Conducted 6 nighttime shipboard CTD hydrocasts to 300-m depth as part of an offshore transect.
- 11 September Continued operations at Pearl and Hermes Atoll. Established 2 NCRMP sites and retrieved existing ARMS, CAUs, STRs, and 1 EAR. Four CRED scientists conducted a Crown of Thorns (*Acanthaster planci*) survey/search within the lagoon area, but were unable to find a single specimen. Overnight transit to Lisianski Island.
- 12 September Arrived Lisianski Island ~ 0600, commenced field operations. Established 2 NCRMP sites and retrieved existing ARMS, CAUs, STRs, and 1 EAR. Conducted 6 nighttime shipboard CTD hydrocasts to 300-m depth as part of an offshore transect.
- 13 September Continued operations at Lisianski Island. Established 2 NCRMP sites and retrieved existing ARMS, CAUs, and STRs. Conducted 5 nighttime shipboard CTD hydrocasts to 300-m depth as part of an offshore transect. As a result of good weather and faster than planned transit speeds, the *Hi'ialakai* cruise schedule was 1 day ahead. In 2006 CRED deployed an STR at Gardner Pinnacle and had not been able to since return to that location for an instrument recovery. Permission was granted from PMNM to conduct an instrument recovery dive at Gardner Pinnacle and even though this location was not on the original ship schedule, the ship's command began a transit to Gardner Pinnacle.
- 14 September Transit to Gardner Pinnacle. Processed/photographed/sorted the ARMS which had been recovered from Pearl and Hermes Atoll and Lisianski Island.
- 15 September Arrived Gardner Pinnacle ~ 1100. The weather did not allow for small boat/diving operations. STR recovery dive cancelled. Processed/photographed/sorted the ARMS which had been recovered from Pearl and Hermes Atoll and Lisianski Island. Transit to O'ahu Island.
- 16 September Transit to O'ahu Island. Processed/photographed/sorted the ARMS which had been recovered from Pearl and Hermes Atoll and Lisianski Island.



- 17 September Arrived O`ahu Island ~ 2300. Still 1 day ahead of the *Hi`ialakai* cruise schedule, the scientific party decided to dive O`ahu Island's west side (a task that was to occur in October 2013 during a land-based mission) on the 18<sup>th</sup> to take advantage of the field assets, personnel/small boats/gear, onboard the *Hi`ialakai*.
- 18 September On station, O`ahu Island, commenced field operations. Established 1 NCRMP site, took on 2 land based CRED divers (for the day) to conduct a full suite of Rapid Ecological Assessment (REA) dives on the west side of O`ahu Island.
- 19 September Transited to and arrived at Pearl Harbor, Honolulu, Hawaii. Disembarked all scientific personnel. End of HA1305.

NOAA's Coral Reef Conservation Program (CRCP) has made the strategic decision to invest a portion of its annual operating budget in perpetuity to support a NCRMP designed to provide a consistent flow of information to assess and report the status and trends of environmental conditions, living reef resources, and the people and processes that interact with coral reef ecosystems. The NCRMP builds on a decade of CRCP-supported, nationwide coral reef monitoring and reporting efforts, such as the Pacific Reef Assessment and Monitoring Program (Pacific RAMP), a CRED-led research program, and *The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States*, a NOAA Technical Memorandum compiled by the NOAA Center for Coastal Monitoring and Assessment. Although the scope of NCRMP is broad, it is intended to assess the status of coral reef ecosystems and their conditions throughout U.S. states and territories and provide a steady and comprehensive analytical context to gauge changes in conditions at the subjurisdictional scale of an island or atoll.

The NCRMP focuses on four priority themes: climate change and ocean acidification; benthic communities (especially corals); reef-associated fish communities; and socioeconomics. Biological monitoring for benthic and fish communities are conducted using a two-stage stratified random sampling design throughout shallow-water (0–30 m), hard-bottom coral reef habitats. Monitoring of climate change and ocean acidification is achieved by means of sustained, remotely sensed and in situ observations of ocean temperature; autonomous, discrete water sampling for analyses of carbonate chemistry; and distinct biological installations designed to provide integrated, ecosystem-wide response data (e.g., biodiversity, calcification, and bioerosion) in the context of climate change. In the Pacific, biological (benthic and fish) and climate monitoring are conducted on a triennial basis. Socioeconomic monitoring is led by the CRCP at headquarters in Silver Spring, MD, and stands outside the scope of the NCRMP monitoring and assessment cruises; therefore, it is not addressed in this cruise report.



## MISSIONS:

HA-13-05 was a unique research cruise in that it was scheduled to only conduct the climate change and ocean acidification portion of NCRMP. FY13 ship time was greatly reduced in the Northwestern Hawaiian Islands and it was deemed that the resulting days on station was too little time for the biological component of CRED's Reef Assessment and Monitoring Program (RAMP) to be conducted in a way to be statistically relevant.

Conducted ecosystem monitoring for benthic cover (community structure), fish populations (species composition, abundance and size), coral populations (species composition, abundance, size distribution, and condition), and target invertebrates of the shallow-water ( $\leq 30$  m) coral reef ecosystems of the main Hawaiian Islands.

- A. Deployed and retrieved a suite of instruments and installations—including STRs, CTD sensor, ARMS, CAUs, BMUs, and EARs—to allow for remote, long-term monitoring of oceanographic, environmental, and ecological conditions of the coral reef ecosystems of the main Hawaiian Islands.
- B. Conducted shallow-water CTD casts and collected water samples for DIC, TA, salinity, and microbial community analyses to depths  $\leq 30$  m to examine physical and biological linkages supporting and maintaining these island ecosystems.
- C. Conducted shipboard oceanographic and meteorological observations to examine physical and biological linkages supporting and maintaining these island ecosystems, using CTD casts deployed to a depth of 500 m with concurrent water samples taken at select locations and depths, collecting continuous acoustic Doppler current profiler (ADCP), sea-surface temperature (SST), and salinity, ecosystems and fundamental meteorological data, such as air temperature, wind speed and direction, barometric pressure, and relative humidity.
- D. Collected a small number of shallow-water coral cores to examine calcification (growth) rates in recent decades and assess potential early impacts of ocean acidification.
- E. Collected a small number of shallow-water rubble samples to examine the microbes
- F. Determined the existence of threats to the health of these coral reef resources from anthropogenic sources, including marine debris.

## RESULTS:

This section provides tallies of research activities (Table 1), a list of data collected during cruise HA-13-05, and a summary of important observations. For more information pertaining to the data collected, methodology employed at the islands visited, see Appendices A–H.

**Table 1.** Statistics for the Pacific RAMP 2013 cruise to the Northwestern Hawaiian Islands (cruise HA-13-05), including the islands/atolls of Pearl and Hermes Atoll (PHR), Lisianski Island (LIS), French Frigate Shoals (FFS), and O`ahu (OAH).

Research Activity	FFS	LIS	OAH	PHR	TOTAL
Scuba Dives	128	92	56	105	138
Biological Surveys					
REA Sites: Benthic	0	0	9	0	9
REA Sites: Fish	0	0	11	0	11
Oceanography Benthic Survey	10	7	0	8	25
Biological Sample Collections					
Bivalve Sand Collection	11	9	0	14	34
Microbial Water Samples	12	9	0	10	31
Microbial Benthic Samples	0	2	0	0	2
Biological Monitoring Installations					
ARMS Retrieved	15	11	0	7	33
ARMS Deployed	12	0	3	12	27
CAUs Retrieved	25	17	0	21	63
CAUs Deployed	25	25	5	25	80
BMUs Deployed	25	0	5	25	55
EARs Retrieved	2	1	0	2	5
Oceanographic Moored Instruments					
SST Buoys Retrieved	0	1	0	0	1
STRs Retrieved	8	6	0	8	22
STRs Deployed	16	15	3	12	46



**Table 1** (continued)

Research Activity	FFS	LIS	OAH	PHR	TOTAL
WTRs Retrieved	0	1	0	0	1
<b>Hydrographic Surveys</b>					
Shallow-water CTD Casts	14	11	4	12	41
Deepwater CTD Casts: Total	12	11	6	6	29
Total Length (km) of ADCP Transects	20.1	18.64	9.88	10.62	59.24
<b>Water-quality Sampling</b>					
Shallow-water Salinity Water Samples	18	15	5	16	54
Shallow-water DIC Water Samples	18	15	5	16	54
Deepwater Chlorophyll- <i>a</i> Water Samples	60	55	0	30	145

The coral reef ecosystems of the Northwestern Hawaiian Islands have been surveyed biennially since 2002 through CRED's Pacific RAMP. The cruise HA-13-05 marked this program's sixth expedition to the region, but CRED's first effort to implement the climate portion of NCRMP. Herein, we present highlights, by island, from our observations during this latest expedition:

#### French Frigate Shoals

- Established a complete installation of the NCRMP, with STR transects in the N, S, E, and W.
- Recovered 15 ARMS from previous deployments, significantly adding to the body of knowledge regarding benthic cryptic biota in the region.

#### Pearl and Hermes Atoll

- Established a complete installation of the NCRMP, with STR transects in the N, S, E, and W.
- Discovered, photograph/video documented, and reported on a very large derelict fishing net along the atoll's northwestern forereef to the Pacific Islands Fisheries Science Center (PIFSC) and the National Ocean Service Pacific Coastal Management and Marine Debris office. Unable to recover the net with the resources on hand, the net was marked with a satellite tag donated by the PIFSC Protected Species Division.

#### **Lisianski Island**

- Established a complete installation of the NCRMP, with STR transects in the N, S, E, and W.

#### **O`ahu Island**

- Good weather and quicker transit times returning from the Northwestern Hawaiian Islands allowed HA-13-05 to conduct operations on the west side of the island and CRED scientists to initiate their O`ahu RAMP, scheduled for October 2013.

### **Climate and Ocean Acidification Monitoring**

#### ***Oceanographic Instrumentation and Biological Installations***

- Seawater temperature at 1, 5, 15, 25 m depths.
- Assessment of taxonomic diversity of cryptic coral reef species by collection of invertebrate specimens from retrieved ARMS.
- Installation of CAUs, providing for a future assessment of calcification rates once the CAUs are retrieved in 2016.
- Installation of BMUs, providing for a future assessment of bioerosion rates once the BMUs are retrieved in 2016.

#### ***Nearshore Oceanography from Small Boats***

- Shallow-water CTD hydrocasts to depths up to 30 m.
- Collection of discrete water samples, collected in concert with shallow-water ( $\leq 30$  m) CTD hydrocasts. Samples are collected for later analysis of DIC, TA, and precision salinity.

#### ***Shipboard Oceanography***

- CTD rosette hydrocasts to a depth of 300 m.
- Collection of discrete water samples, collected in concert with deepwater ( $> 150$  m) CTD rosette hydrocasts. Samples are collected for later analysis of Chlorophyll-*a* concentration at variable depths, 0–300 m.
- Dissolved oxygen, turbidity, fluorescence, and pH measurements recorded by sensors integrated into the CTD package.
- Profiles of ocean current direction and magnitude collected using a shipboard ADCP.
- Solar radiation, air temperature, barometric pressure, and wind direction and magnitude collected using shipboard instrumentation.
- Surface seawater temperature and salinity measurements from real-time flow through shipboard instrumentation.



## **Biological Monitoring**

### ***Benthic Surveys***

- Digital still photographs of overall site character and typical benthos.
- Digital still photographs of the benthos along transect lines.
- Number, species or genus, size, and condition of all coral colonies observed within belt transects of known area.
- Digital still photographs of diseased corals and coralline algae.
- Water samples and benthic rubble grabs at select REA sites for microbial analyses.

### ***Fish Surveys (O`ahu only)***

- Number, species, and estimated sizes of all fishes observed within visually estimated 7.5 m-radius, stationary-point-count surveys.
- Visual estimates of benthic cover, habitat type, and habitat complexity.
- Digital still photographs of the benthos along transect lines.
- Digital still photographs of rare or interesting fish species.
- Species presence checklists for estimates of fish community diversity.

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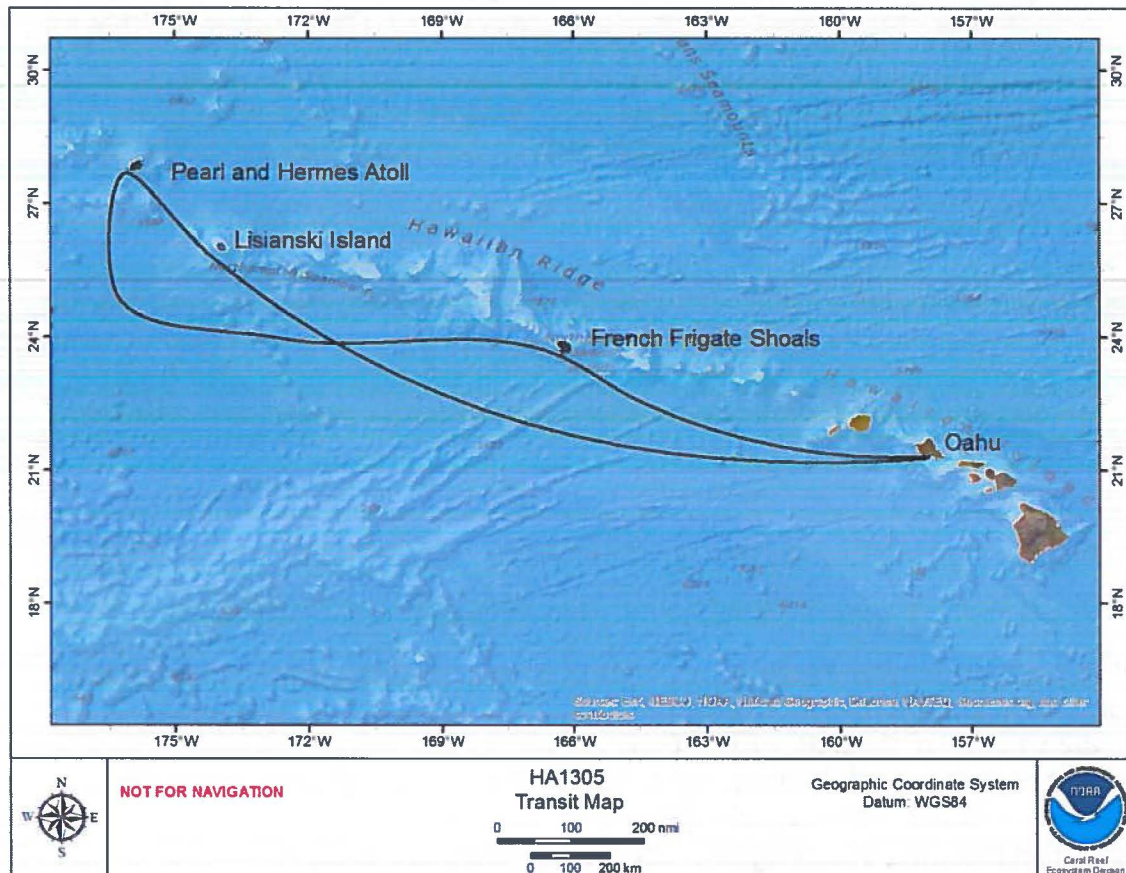
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**Figure 1.** Track of the NOAA Ship *Hi'ialakai* for the cruise HA-13-05, September 3–19, 2013, with French Frigate Shoals, Pearl and Hermes Atoll, Lisianski Island, and O'ahu Island surveyed. Satellite image: SIO, NOAA, U.S. Navy, NGA, GEBCO (Becker, 2009; Smith and Sandwell, 1997) © 2008 The Regents of the University of California.



## APPENDIX A: METHODS

This appendix describes the methods and procedures used by the Coral Reef Ecosystem Division (CRED) of the NOAA Pacific Islands Fisheries Science Center during its Pacific Reef Assessment and Monitoring Program (Pacific RAMP) cruise HA-13-05 on the NOAA Ship *Hi'ialakai* during the September 3–19, 2013, period.

### **A.1. Climate and Ocean Acidification Monitoring: Instrumentation, Biological Installations, and Water Quality**

Activities conducted for the monitoring of climate and ocean change: (1) nearshore oceanographic and water quality surveys; (2) deployment and retrieval of an array of subsurface moored instrumentation and installations selected to provide continuous, high-resolution time series of physical observations or integrated, ecosystem-wide biological process data; (3) offshore oceanographic surveys characterizing physical, biological, and chemical water properties, and ocean currents around these islands; and (4) shipboard meteorological observations, including wind speed and direction, relative humidity, air temperature, and barometric pressure. In addition, previously deployed instrumentation such as ecological acoustic recorders (EARs), which monitor the sounds of marine animals and vessel traffic around the islands, were also retrieved.

Monitoring of climate and ocean acidification at each survey site falls into four different levels of increasing scientific investigation. These efforts are intended to document island-scale, water column chemistry, the spatial and temporal variability of near-reef waters thermal structure across a depth gradient, and the integrated biological responses of the reef community to climate change.

- 1- Class-0 sites: Only discrete water samples are collected and analyzed for DIC and TA.
- 2- Class-1 sites: Only subsurface temperature recorders (STRs), SBE 56 temperature loggers (Sea-Bird Electronics, Inc., Bellevue, Wash.), are deployed.
- 3- Class-2 sites: Include collection of discrete water samples for DIC and TA, STR deployments, benthic surveys and still photographic records of the benthos. Installations related to climate studies include calcification accretion units (CAUs), bioerosion monitoring units (BMUs), and autonomous reef monitoring structures (ARMS). Coral coring is conducted at these sites as well.
- 4- Class-3 sites: A MAP pCO<sub>2</sub> buoy system is added to the setup for a Class-2 site.

Most of the CRED's efforts focus on establishing Class 0–2 sites at select locations distributed along the four cardinal directions around each island surveyed. Thermal structure measurements are obtained through the deployment of subsurface temperature recorders (STRs; SBE 56) along a perpendicular to shoreline forereef transect at 1, 5, 15, and 25-m depth; each SBE 56 records the near-reef water temperature at the same time,

on a 5-min interval, for the duration of the instrument's deployment. Within this context, at select areas, a permanent water quality, temperature, and biological survey/sampling site, designated as *NCRMP Survey Site*, is established at the 15-m depth STR location. The NCRMP Survey Site includes: deployment of 1 STR, 3 ARMS units, 5 CAUs, and 5 BMUs; collection of 3 carbonate chemistry water samples (with associated CTD casts); acquisition of still photographic benthic imagery to document benthic cover and composition; and rugosity measurements of benthic topographic complexity.

#### **A.1.1. Moored Instruments for Time-series Observations**

CRED accomplishes long-term oceanographic assessment and monitoring through the deployment and retrieval of a variety of platforms, which either electronically record *in-situ* measurements (temperature, currents, and waves) or by facilitating biological recruitment/growth on fabricated structures. The following types of oceanographic instruments and biological installations were retrieved or deployed during this cruise:

**Subsurface Temperature Recorder (STR):** provides high-resolution temperature data (SBE 39 and SBE 56). Data are internally recorded at 5-min intervals with SBE 56 and at 30-min intervals with the SBE 39. This type of subsurface instrument is deployed at depths of 1-25 m. All loggers retrieved during HA-13-05 were of the type SBE 39; all loggers deployed were of the type SBE 56.

**Calcification Accretion Unit (CAU):** are fabricated structures used to detect changes in calcification rates and net accretion of crustose coralline algae and other benthic sessile calcifiers.

**Bioerosion Monitoring Unit (BMU):** are calcium carbonate fragments which serve as a proxy for an integrated signal of net reef bioerosion.

**Autonomous Reef Monitoring Structure (ARMS):** provides an assessment of cryptic taxonomic diversity of coral reef associated species.

#### **A.1.2. Hydrographic Surveys**

Detailed oceanographic and water quality surveys were conducted using the following sampling techniques and equipment.

**Shallow-water (Nearshore) Conductivity, Temperature, and Depth Hydrocasts:** a CTD profiler (SBE 19*plus* SeaCAT Profiler) deployed from a small boat provides water column data on temperature, conductivity (which is related to salinity) and pressure (which is related to depth). A transmissometer (C-Star, WET Labs, Philomath, Ore.) provided profiles of beam transmittance and related to water-column turbidity. A dissolved oxygen sensor (SBE 43, accuracy of 2% of saturation) also is attached to the CTD.

A CTD cast was performed at each location where a water sample was collected. The CTD is lowered by hand, off a small boat at descent rates of ~ 0.5–0.75 m/sec to depths up to 30 m.



**Deepwater (Shipboard) CTD Hydrocasts:** a ship-based CTD profiler provide high-resolution conductivity, temperature, and pressure data (SBE 911plus CTD, accuracy of 0.003 S m<sup>-1</sup> in conductivity, 0.001°C in temperature, and 0.015% in pressure). Measurements of dissolved oxygen (SBE43) and fluorescence and turbidity (ECO FLNTU, WET Labs, accuracy of 0.01 µg/L in fluorescence and 0.01 NTU in turbidity) were performed in concert with CTD measurements. Data were collected at depths up to 300 m.

**Shipboard Acoustic Doppler Current Profiler (ADCP):** a ship-based sensor provided transects of directional ocean current data (75-kHz Ocean Surveyor, Teledyne RD Instruments Inc., Poway, Calif.). The system was configured with an 8-m pulse length, 16-m depth bins starting at 25 m and extending typically to 600 m (range depended on density and abundance of scatterers), and 15-min averaged ensembles.

**Water Chemistry:** water samples for analyses of concentrations of Chlorophyll-*a* (Chl-*a*), dissolved inorganic carbon (DIC), and Total Alkalinity (TA), were collected at select locales concurrently with CTD hydrocasts.

## **A.2. Biological Monitoring: Benthic Surveys and Microbial Sampling**

*(Conducted in-full on the west side of O`ahu Island and partially during the NW Hawaiian Islands RAMP, which focused on the climate portion of NCRMP)*

A two-stage stratified random sampling design was employed to survey the Rapid Ecological Assessment (REA) sites in the main Hawaiian Islands Archipelago. The survey domain encompassed 99.6% of the mapped area of reef and hard bottom habitats and was divided into strata based on island, habitat structure type, and depth. The broad habitat structure types included simple, complex, and coral-rich. Depth categories of shallow (0–6 m), moderate (> 6–18m) and deep (> 18–30 m) were also incorporated into the stratification scheme. Allocation of sampling effort was proportional to strata area. Sites were randomly selected within each stratum.

### **A.2.1. Benthic composition and coral demographics**

Surveys at each site were conducted within two 18-meter belt transects. Adult coral colonies ( $\geq 5$  cm) were surveyed within four (1.0 × 2.5 m) segments in the following manner: 0–2.5 m (segment 1); 5.0–7.5 m (segment 3); 10–12.5 m (segment 5); and 15–17.5 m (segment 7). All colonies whose center fell within 0.5 m on either side of each transect line were identified to lowest taxonomic level possible (species or genus), measured for size (maximum diameter to nearest cm), and morphology was noted. In addition, partial mortality and condition of each colony was assessed. Partial mortality was estimated as percent of the colony in terms of old dead and recent dead and the cause of recent mortality was identified if possible. The condition of each colony including disease and bleaching was noted along with the extent (percent of colony affected) and level of severity (range from moderate to acute).

Juvenile coral colonies (< 5 cm) were surveyed within three (1.0 × 1.0 m) segments along the same two transects: 0–1.0 m (segment 1); 5.0–6.0 m (segment 3); and 10.0–11.0 m (segment 5). Juvenile colonies were distinguished in the field by a distinct tissue and skeletal boundary (not a fragment of larger colony). Each juvenile colony was identified to lowest taxonomic level (genus or species) and measured for size by recording both the maximum and perpendicular diameter to the nearest 2 mm.

Still photographs were collected to record the benthos at predetermined points along the same 2 transect lines with a high-resolution digital camera mounted on a pole. Photographs were taken every 1 m, starting at 1 m to the 15-m mark. This work generates 30 photographs per site which are later analyzed by CRED staff and partners, implementing the computer program Coral Point Count with Excel extensions (CPCe), as the basis to estimate the benthic cover and composition at each site (benthic habitat photographs at sites surveyed by the fish team are also be analyzed).

### **A.2.2. Microbial Communities**

Microbes are a fundamental aspect of all marine ecosystems. Trophic-level interactions within the marine microbial food web can have a big effect on global nutrient and carbon cycling. Within a reef system, the amount of energy from primary production that is remineralized by the microbial fraction determines the amount of energy available for the entire food web. Shifts in the abundance and community composition of the microbial community in a reef system have also been linked to declines in coral health.

It is well known that bacteriophages (bacterial viruses) are the most abundant form of life in the ocean, ranging from  $1 \times 10^6$  virus-like particles (VLPs) per mL of seawater in the open ocean to  $1 \times 10^8$  VLPs per mL in more productive coastal waters. The number of microbial cells in seawater is typically  $1 \times 10^6$  cells per mL. Microbial and viral loading and the dominance of heterotrophic bacteria in reef water are linked to coral disease. One of the most direct methods for assessing and monitoring changes in abundance of these microbiological components is by fluorescent microscopy using nucleic acid staining.

A direct parallel exists between microbial and viral loading, increasing human disturbance, and reef health. Microbial communities in more degraded coral reef systems support a high abundance of potential coral pathogens and heterotrophic microbes (a heterotrophic organism obtains food only from organic material, such as carbon and nitrogen, and is unable to use inorganic matter to form proteins and carbohydrates). In contrast, near-pristine reefs support microbial communities that are balanced between heterotrophs and autotrophs and contain very few potential pathogens (an autotrophic organism can synthesize food from inorganic material).

Spatial assessment of microbial and viral components with respect to levels of dissolved organic carbon (DOC), nutrients ( $\text{NO}_2$ ;  $\text{NO}_3$ ;  $\text{NH}_4$ ; and  $\text{PO}_4^{3-}$ ), and particulate organic carbon (POC) within coral reef ecosystems may identify important predictors of coral reef ecosystem degradation. For example, in addition to microbial abundance, bacterial growth efficiency (BGE) may also play a role in reef system health. BGE is affected

greatly by DOC:Nitrogen ( $\text{NO}_x + \text{NH}_4$ ) ratios in the water column. Water-column stoichiometry (C:N:P ratios) directly affect microbial growth rates.

In summary, no long-term data on the dynamics of natural bacterial assemblages in reef systems (let alone other ecotypes) are currently available. Building a pan-Pacific microbial data set is an extremely important step towards greater understanding of the overall health of the reef system. The majority of reefs on the planet are affected and analyses are confounded by the inability to attribute differences in reef system dynamics to variation in resource availability caused by oceanography or human activity. The region monitored through Pacific RAMP includes reefs experiencing various combinations of human activity and resource availability. The hope is that new patterns in the microbial data sets will emerge at regional or pan-Pacific scales and that this information can be used to understand the mechanisms underlying reef system decline.

**Collection of Microbial Water Samples:** As part of the ongoing effort to understand the microbial community, two types of water samples were collected. The first type included two diver-deployable Niskin bottles that were used to collect water at each moderately deep REA site. The Niskin bottles (two 2-L replicates) were filled with “reef water” collected from < 1 m above the benthos. These water samples were returned to the ship and processed for DOC, particulate organic matter (POM), nutrients, microbial (Bacteria and Archaea) and viral counts (fluorescent microscopy), fluorescence-activated cell sorting (FACS, heterotrophs vs autotrophs), and microbial and viral community composition (coarse analysis: 16s rRNA).

The other type of water collection was for metagenomic analysis of the microbial and viral community associated with reef benthos. Only one sample per island was procured. This collection involved carboys (four 20-L replicates) that were also filled with “reef water” collected from < 1 m above the benthos. The samples were collected using a flexible, plastic hose with a carboy bottle attachment. The carboys were filled using a small, lightweight pump attached to the other end of the hose. All microbial collections were conducted at select REA sites (locations with supporting fish or benthic data).

Attempts were made to collect the following data items, daily, at each moderately deep REA site (for reef- and pore-water samples):

- DOC: 2 replicates
- POM: 2 replicates
- Nutrients: 2 replicates
- Microbial (Bacteria and Archaea) and viral abundance: 2 replicates (0.02- $\mu\text{m}$  filters, stained using SYBR Gold, Molecular Probes Inc., Eugene, Ore.)
- Microbial (Bacteria and Archaea) size structure : 2 replicates (0.2- $\mu\text{m}$  filters, stained using 4', 6-Diamidino-2-phenylindole (DAPI))
- Microbial community composition (FACS, heterotrophs/autotrophs): 6 replicates
- Microbial community composition (16s rRNA): 1 (0.22- $\mu\text{m}$  filters)



The following data items were collected once per island at REA sites:

- Microbial community composition (metagenome): 1 sample, (3–6 filters of 0.45  $\mu\text{m}$ )
- Viral community composition (metagenome): 1 sample, (3–6 vials)
- Coral rubble or sediment: 6 replicate bags

**Processing of Water Samples:** This section describes the techniques used to process the water samples.

*Enumeration of microbes and viruses.* Samples of 1 mL from each Niskin were fixed using paraformaldehyde and stained using the general nucleic acid stain SYBR Gold. The samples were filtered through 0.02- $\mu\text{m}$  Anodisc filters and mounted on a microscope slide. Direct counts of microbes and VLPs will be completed using fluorescent microscopy and Image Pro software.

*Microbial biomass.* Samples of 1 mL from each Niskin were fixed using glutaraldehyde and filtered through 0.2- $\mu\text{m}$  filters. These filters were stained with DAPI, a general nucleic acid stain for staining double-stranded DNA (dsDNA) that allows length and width data to be obtained for individual microbes. These filters were then mounted on a microscope slide for analysis using fluorescent microscopy and Image Pro software. Slide analysis will be performed at San Diego State University (SDSU). All filters were stored at  $-20^{\circ}\text{C}$  for archival purposes.

*Enumeration of autotrophic vs. heterotrophic microbes:* Flow cytometry will be used to assess the ratio of autotrophic to heterotrophic microbes in the water column. This technique also will provide complementary data for microbial abundance, microbial community structure, and levels of Chl-*a*.

Six 1-mL samples of water from each REA site were pushed through a 20- $\mu\text{m}$  filter. This filtrate was dispensed into cryovials ( $6 \times 1\text{ mL}$ ) and fixed with glutaraldehyde. Vials were inverted to mix. Glutaraldehyde-preserved samples were flash frozen in liquid nitrogen contained in a dry shipper to prevent damage to microbial cells. These samples will be shipped on dry ice to SDSU for flow cytometry analysis.

*Water Chemistry (DOC/POC):* 30 mL of seawater were filtered through a 47-mm pre-combusted glass fiber filters from each of the 2 Niskin bottles, and the filtrate was collected in pre-combusted plastic bottles. The bottles were stored at  $-20^{\circ}\text{C}$ . To assess POC, 500 mL of seawater were filtered through a 47-mm pre-combusted glass fiber filter (2 replicates), and the filters were stored at  $-20^{\circ}\text{C}$ . Stable isotopes of carbon and nitrogen also will be analyzed from the filters via standard protocols at SDSU.

*Collection of DNA for metagenomics:* The community structure of the microbes and viruses associated with the water column was assessed by metagenomic analysis. Metagenomics is a powerful tool for studying environmental populations as  $< 1\%$  of all environmental microbial diversity is currently cultivable. The steps for analysis of

microbial community diversity and function involve collection of environmental DNA followed by 16S rRNA gene sequencing. Approximately 1.5–2 L of reef water was filtered through a 0.22  $\mu\text{m}$  sterivex filter. DNA isolation and metagenomic analysis will be completed at SDSU.

At one REA site per island, four 20-L collapsible carboys of water were filled with water from reef crevices or reef benthos using a manual bilge pump. Upon return to the ship, this water sample was pre-filtered through 100- $\mu\text{m}$  mesh and concentrated using tangential flow filtration (TFF). TFF concentrates the bacteria and viruses in the water, bringing the initial 70–80 L of water to a final volume of  $\sim 500$  mL. This concentrate was then filtered through 0.45- $\mu\text{m}$  filters to capture microbes (Bacteria and Archaea). These filters were frozen at  $-20^{\circ}\text{C}$ . The DNA of the entire community will be extracted and sequenced at SDSU, and the diversity and function of the microbial communities associated with the reef benthos will be analyzed. The filtrate from this sample contains concentrated viruses. Chloroform was added to this filtrate to kill any small microbes that passed through the 0.45- $\mu\text{m}$  filter, and the sample was stored at  $4^{\circ}\text{C}$ . Once shipped to SDSU, viruses will be isolated from the viral concentrate, and community DNA will be extracted and sequenced. This extracted and sequenced DNA will then be analyzed for viral community diversity and function.

**Collection of Benthic Samples (if time permits):** This section describes samples, or benthic grabs, collected if time permitted.

*Collection of benthic microbial DNA:* In addition to changes in the microbial community associated with the water column, we are also interested in whether or not community shifts in microbes associated with the benthos are a useful indicator of reef health. When time permits, six “fist fulls” of coral rubble or sediment and six pieces of the most dominant algal-type will be collected in Ziploc bags. Both the algal and rubble/sediment samples were frozen at  $-20^{\circ}\text{C}$ . These samples stayed on the ship until it returned to Honolulu. The bacterial 16s rRNA genes associated with these samples will be sequenced to characterize the microbial communities associated with the benthos (rubble and algae).

### **A.3. Biological Monitoring: Surveys of Reef Fishes**

*(Conducted in-full on the west side of O`ahu Island only)*

Divers conducted REA fish surveys using the stationary-point-count (SPC) method at preselected REA sites. Two separate teams performed these surveys. Each team consisted of 2 divers, and conducted 1 SPC survey per site. All fish REA sites visited were selected using a stratified random sampling design in shallow (0–6 m), moderate (6–18 m), or deep (18–30 m) depth strata, in forereef, backreef, and lagoon habitat strata, when applicable. Surveys were performed using a 30-m transect line set along a single depth contour. The REA sites selected for fish surveys typically differ in location from the REA sites where benthic surveys were conducted.

Once a transect line was deployed, the 2 divers moved to the 7.5-m and 22.5-m marks on this transect line to start their SPC surveys. Each of these marks or points, with 1 diver at

each, served as the center of a visually estimated cylindrical survey area with a radius of 7.5 m. During the first 5 min, divers only recorded the presence of species within their respective cylinders. Afterwards, divers went down their respective species lists, which were created from their work during the initial 5 min of a survey, sizing and counting all individuals within their cylinder, one species at a time. Cryptic species missed during the initial 5 min of a survey could still be counted, sized, and added to the original species list. Fish species observed at a REA site but not recorded during the SPCs were recorded for presence data.

After a survey was completed, divers recorded benthic habitat information within their respective cylindrical survey areas. Divers visually estimated habitat complexity, habitat type, and percentage of cover for hard corals, macroalgae, crustose coralline red algae, turf algae, and sand. Urchin densities were also estimated. Every meter along the transect line, still photographs were taken of the benthos to the right side of the line. Like the photographs taken along transect lines during surveys at REA benthic sites, these images will be analyzed later.



## APPENDIX B: FENCH FRIGATE SHOALS

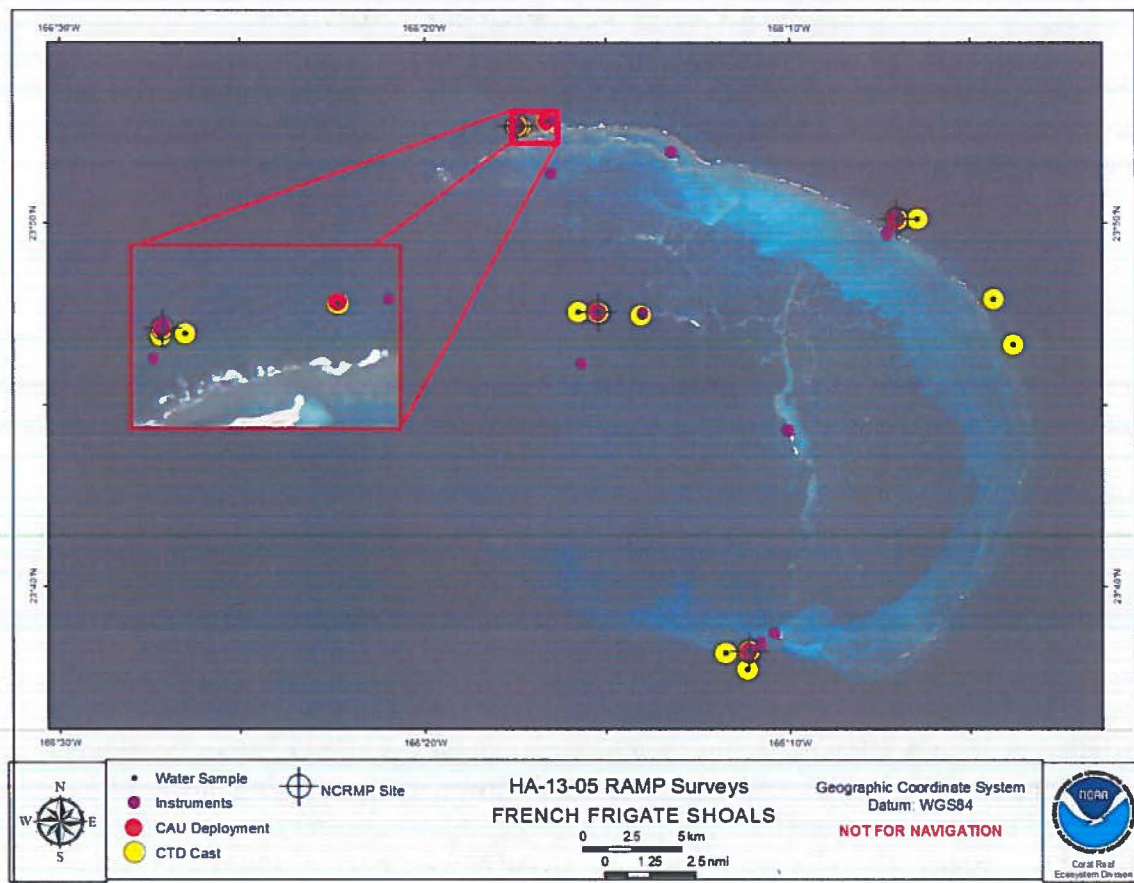
The atoll of French Frigate Shoals is located at 23°44' N, 166°8' W in the north Pacific. For information about the methods used to perform the activities discussed in this appendix, please see Appendix A: "Methods."

### **B.1. Climate and Ocean Acidification Monitoring: Instrumentation, Biological Installations, and Water Quality**

Oceanographic operations during the cruise HA-13-05 at French Frigate Shoals entailed numerous retrievals and deployments of oceanographic moored instruments, installation of subsurface temperature recorders (STRs), calcification acidification units (CAUs), autonomous reef monitoring structures (ARMS), bioerosion monitoring units (BMU), nearshore water sampling and conductivity, temperature, and depth (CTD) casts at select *NCRMP* sites, and shipboard water sampling and CTD casts offshore to a depth of 300 m, and acoustic Doppler current profiler (ADCP) transects.

Fourteen shallow-water CTD casts were performed, one occurring at each location where water samples were collected, this included sample locations taken in concert with the installation of *NCRMP* monitoring stations as well as at stratified random locations around the island. Eighteen shallow-water samples were collected for analysis of dissolved inorganic carbon (DIC), total alkalinity (TA), and salinity. In addition, 8 STRs were retrieved and 16 STRs were deployed. 15 ARMS were recovered and processed for taxonomic analysis and 12 ARMS were deployed. 25 CAUs were recovered and 25 CAUs were deployed at 5 locations around the island. Twenty-five BMUs were collocated at the CAU sites as part of the *NCRMP* survey sites. One EAR mooring was also retrieved (Fig. B.1.1 and Table B.1.1).

From the NOAA Ship *Hi'ialakai*, ~20.1 km of ADCP transect lines were run in the east and west direction, away from this island during night operations. On the reciprocal course, shipboard CTD casts were conducted to a depth of 300 m per cast every ~ 2 km for a total of 12 deep water CTD casts around French Frigate Shoals. Water samples were collected concurrently at 5 depths between the surface and ~ 200 m, depending on the depth of mixed layer as determined by the CTD downcast. (Fig. B.1.2).



**Figure B.1.1.--**Mooring sites where oceanographic instruments and biological installations were retrieved or deployed and locations of nearshore CTD casts and water sampling performed at French Frigate Shoals during cruise HA-13-05. Sources: Landsat satellite imagery data from the U.S. Geological Survey; bathymetry imagery data from: ESRI, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors.

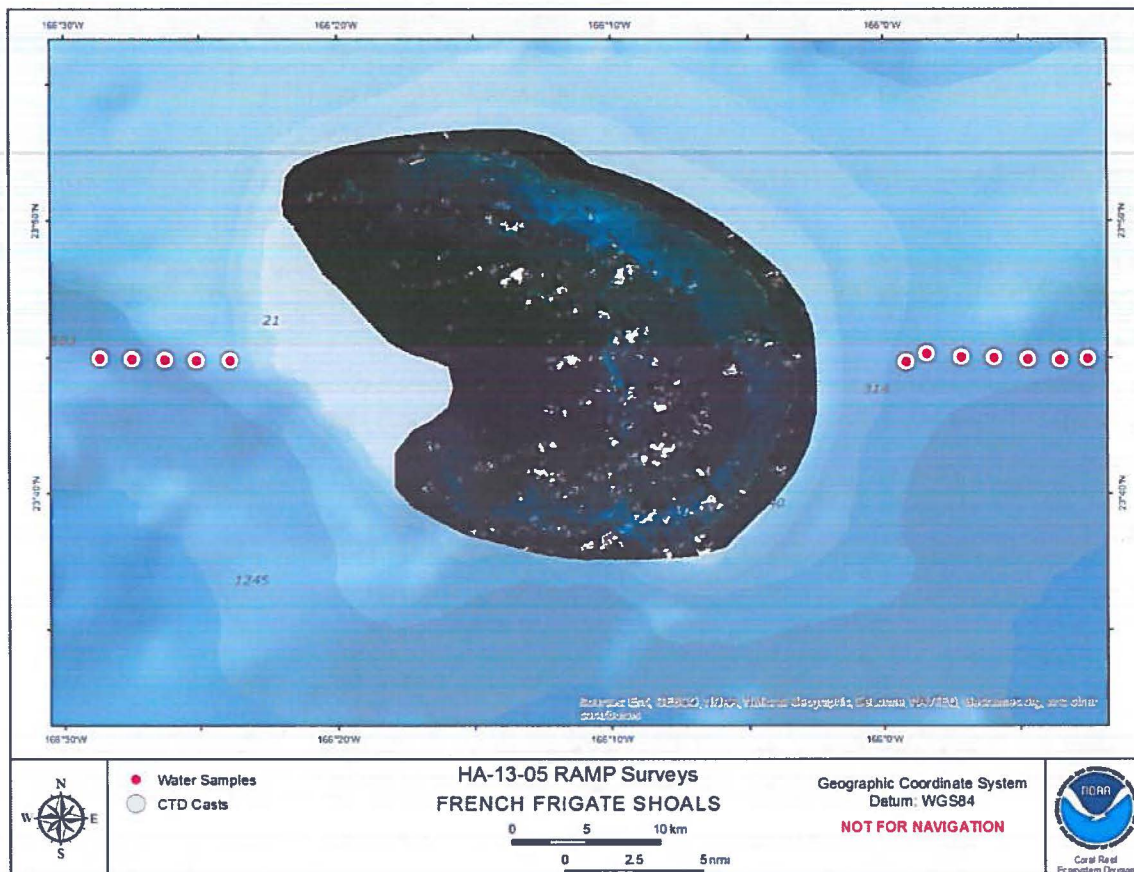
**Table B.1.1.--**Geographic coordinates and depths of the moored oceanographic instruments (STRs and EARS) and biological installations (CAUs, ARMS, and BMUs), that were retrieved or deployed at French Frigate Shoals during cruise HA-13-05.

Site	Date	Instrument Type	Latitude	Longitude	Depth (m)	Retrieved	Deployed
FFS-12	05-Sep-13	ARMS	23.63835	-166.18005	7.9	3	-
FFS-34	05-Sep-13	ARMS	23.62792	-166.13538	10.4	3	-
FFS-12	05-Sep-13	CAU	23.63835	-166.18005	7.9	5	-
FFS-34	05-Sep-13	CAU	23.62792	-166.13538	10.4	5	-
FFS-11	05-Sep-13	EAR	23.63498	-166.18559	24.1	1	-
FFS-11	05-Sep-13	STR	23.63498	-166.18559	24.1	-	1
FFS-32	06-Sep-13	ARMS	23.80610	-166.23063	7.9	3	-
FFS-35	06-Sep-13	ARMS	23.79062	-166.23202	8.8	3	-
FFS-40	06-Sep-13	ARMS	23.63686	-166.18515	13.7	-	3
FFS-41	06-Sep-13	ARMS	23.79247	-166.25372	13.7	-	3
FFS-40	06-Sep-13	BMU	23.63686	-166.18515	13.7	-	5
FFS-41	06-Sep-13	BMU	23.79247	-166.25372	13.7	-	5
FFS-21	06-Sep-13	CAU	23.84695	-166.32695	16.8	5	-
FFS-40	06-Sep-13	CAU	23.63686	-166.18515	13.7	-	5
FFS-41	06-Sep-13	CAU	23.79247	-166.25372	13.7	-	5
FFS-R46	06-Sep-13	CAU	23.76928	-166.26173	8.2	5	-
FFS-10	06-Sep-13	STR	23.63884	-166.17977	11.0	1	-
FFS-12	06-Sep-13	STR	23.85619	-166.27515	7.9	1	-
FFS-15	06-Sep-13	STR	23.63686	-166.18514	13.7	-	1
FFS-16	06-Sep-13	STR	23.64094	-166.17968	6.4	-	1
FFS-17	06-Sep-13	STR	23.79185	-166.23322	4.9	-	1
FFS-18	06-Sep-13	STR	23.79247	-166.25372	13.7	-	1
FFS-2	06-Sep-13	STR	23.76894	-166.26133	4.3	1	1
FFS-3	06-Sep-13	STR	23.86611	-166.21968	3.0	1	1
FFS-4	06-Sep-13	STR	23.64513	-166.17376	1.5	1	1
FFS-5	06-Sep-13	STR	23.73814	-166.16749	2.7	1	1
FFS-6	06-Sep-13	STR	23.85619	-166.27515	0.3	1	-
FFS-33	07-Sep-13	ARMS	23.83647	-166.26683	9.1	3	-
FFS-42	07-Sep-13	ARMS	23.87806	-166.29100	15.2	-	3
FFS-44	07-Sep-13	ARMS	23.83515	-166.11682	15.2	-	3
FFS-42	07-Sep-13	BMU	23.87806	-166.29100	15.2	-	5
FFS-43	07-Sep-13	BMU	23.88021	-166.27707	15.2	-	5
FFS-44	07-Sep-13	BMU	23.83515	-166.11682	15.2	-	5
FFS-42	07-Sep-13	CAU	23.87806	-166.29100	15.2	-	5
FFS-43	07-Sep-13	CAU	23.88021	-166.27707	15.2	-	5



**Table B1.1 (continued)**

Site	Date	Instrument Type	Latitude	Longitude	Depth (m)	Retrieved	Deployed
FFS-44	07-Sep-13	CAU	23.83515	-166.11682	15.2	—	5
FFS-H6	07-Sep-13	CAU	23.88043	-166.27307	12.2	5	—
FFS-14	07-Sep-13	STR	23.88043	-166.27308	8.5	1	—
FFS-19	07-Sep-13	STR	23.82794	-166.12122	2.1	—	1
FFS-20	07-Sep-13	STR	23.83019	-166.12026	5.5	—	1
FFS-21	07-Sep-13	STR	23.83515	-166.11683	16.8	—	1
FFS-22	07-Sep-13	STR	23.83665	-166.11702	26.2	—	1
FFS-23	07-Sep-13	STR	23.87565	-166.29173	6.4	—	1
FFS-24	07-Sep-13	STR	23.87806	-166.29101	16.8	—	1
FFS-25	07-Sep-13	STR	23.87854	-166.29093	25.0	—	1



**Figure B.1.2.--**Locations of deepwater CTD casts and water sampling performed at French Frigate Shoals during cruise HA-13-05. Sources: Landsat satellite imagery data from the U.S. Geological Survey; bathymetry imagery data from: ESRI, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors.

## B.2. Biological Monitoring: Microbial Sampling

Water samples for microbial analyses were collected at 5 REA sites (Table B.2.1). For more information about collections made at REA sites, see Table I.1.1 in Appendix I: “Biological Collections.”

**Table B.2.1.**--Summary of microbial water collections performed at French Frigate Shoals during cruise HA-13-05.

REA Site	Date	Reef Zone	Depth	Latitude	Longitude	Microbial Samples
FFS-1006	06-Sep-13	Forereef	13.7	23.79	-166.25	3
FFS-40	06-Sep-13	Forereef	13.7	23.64	-166.19	2
FFS-42	07-Sep-13	Forereef	15.2	23.88	-166.29	3
FFS-43	07-Sep-13	Forereef	15.2	23.88	-166.28	2
FFS-44	07-Sep-13	Forereef	15.2	23.84	-166.12	2





## APPENDIX C: PEARL AND HERMES ATOLL

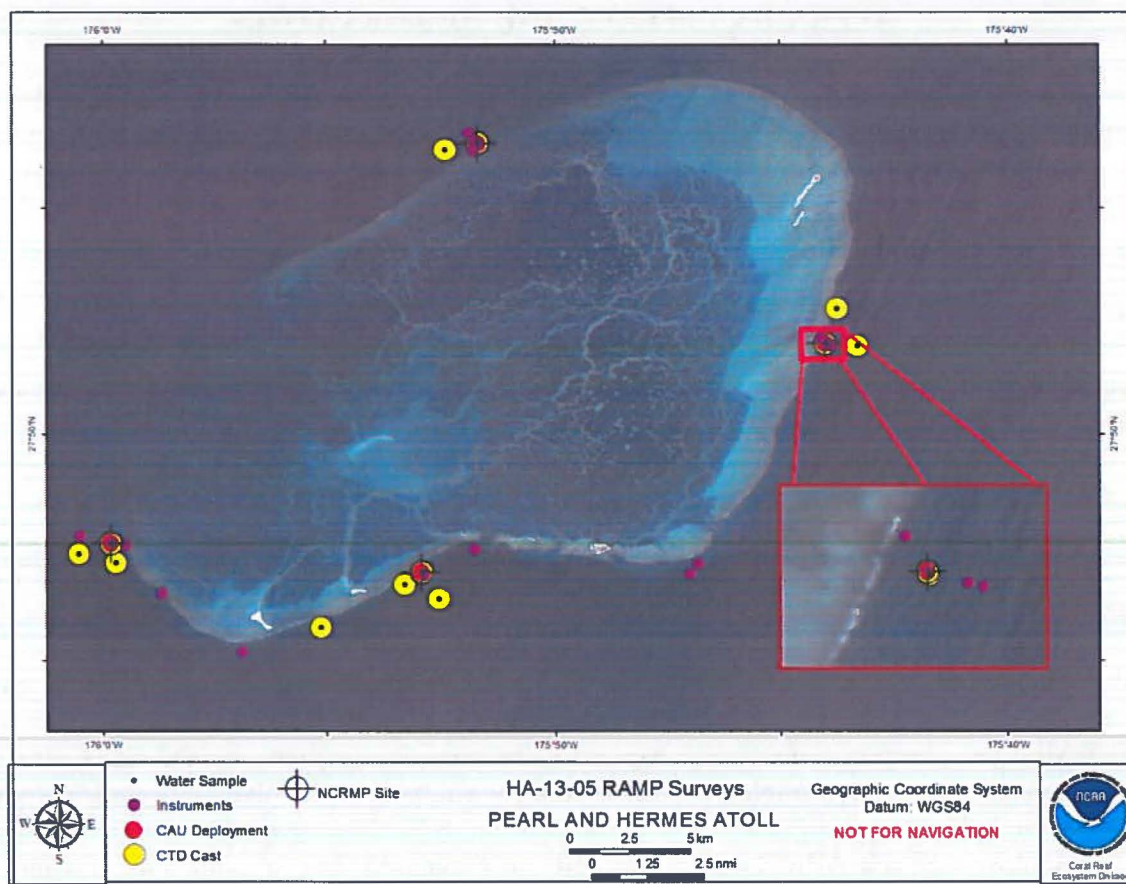
Pearl and Hermes Atoll is located at 27°48' N, 175°51' W in the north Pacific and is the northernmost location that HA-13-05 surveyed. For information about the methods used to perform the activities discussed in this appendix, please see Appendix A: "Methods."

### **C.1. Climate and Ocean Acidification Monitoring: Instrumentation, Biological Installations, and Water Quality**

Oceanographic operations during the cruise HA-13-05 at Pearl and Hermes Atoll entailed numerous retrievals and deployments of oceanographic moored instruments, installation of subsurface temperature recorders (STRs), calcification acidification units (CAUs), autonomous reef monitoring structures (ARMS), bioerosion monitoring units (BMUs), nearshore water sampling and conductivity, temperature, and depth (CTD) casts at select *NCRMP* sites, and shipboard water sampling and CTD casts offshore to a depth of 300 m, and acoustic Doppler current profiler (ADCP) transects.

Thirteen shallow-water CTD casts were performed at each location where water samples were collected, this included sample locations taken in concert with the installation of *NCRMP* monitoring stations as well as at stratified random locations around the island. Eighteen shallow-water samples were collected for analysis of dissolved inorganic carbon (DIC), total alkalinity (TA), and salinity. In addition, 8 STRs were retrieved and 12 STRs were deployed. Seven ARMS were recovered and processed for taxonomic analysis and 12 ARMS were deployed. 21 CAUs were recovered and 25 CAUs were deployed at 5 locations around the island. Twenty-five BMUs were collocated at the CAU sites as part of the *NCRMP* survey sites. Two EARs mooring were also retrieved (Fig. C.1.1 and Table C.1.1).

From the NOAA Ship *Hi'ialakai*, ~10.6 km of ADCP transect lines were run in the east and west direction, away from this island during night operations. On the reciprocal course, shipboard CTD casts were conducted to a depth of 300 m per cast every ~ 2 km for a total of 6 deep-water CTD casts around Pearl and Hermes Atoll. Water samples were collected concurrently at 5 depths between the surface and ~ 200 m, depending on the depth of mixed layer as determined by the CTD downcast. (Fig. C.1.2).



**Figure C.1.1.--**Mooring sites where oceanographic instruments and biological installations were retrieved or deployed and locations of nearshore CTD casts and water sampling performed at Pearl and Hermes Atoll during cruise HA-13-05. Sources: Landsat satellite imagery data from the U.S. Geological Survey; bathymetry imagery data from: ESRI, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors.

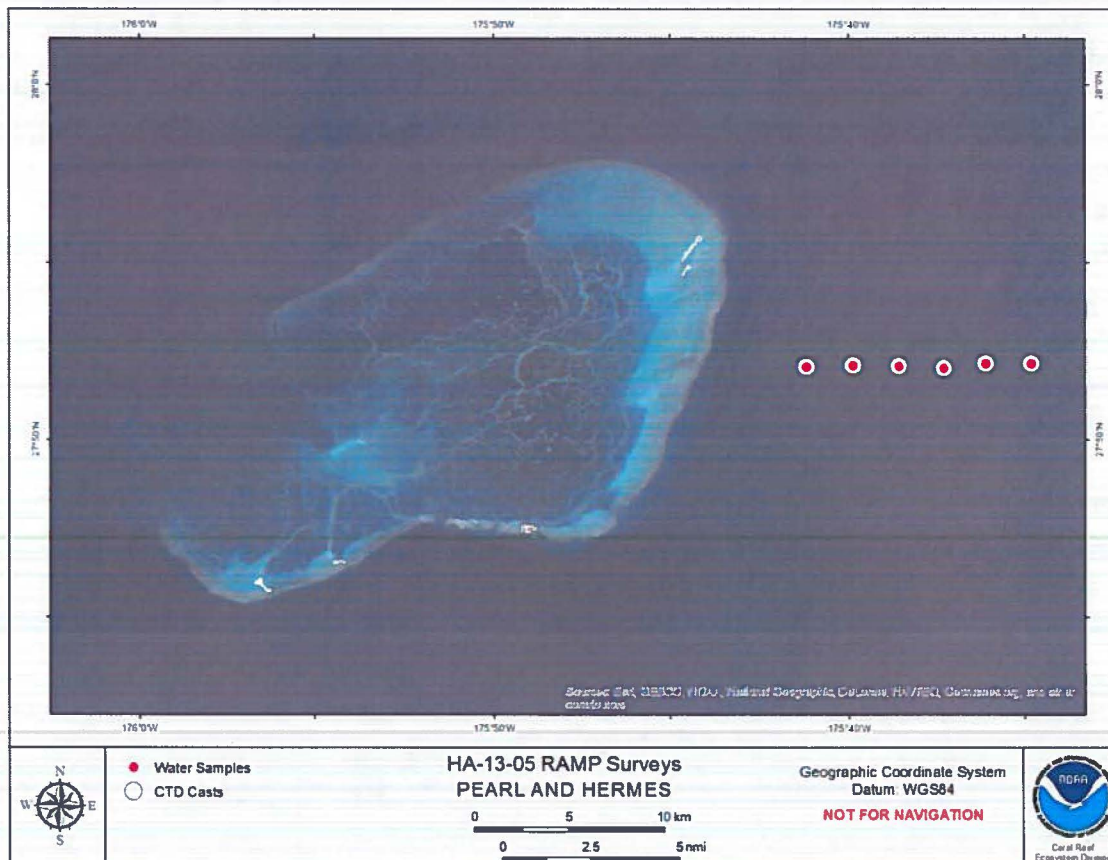
**Table C.1.1. --** Geographic coordinates and depths of the moored oceanographic instruments (STRs and EARs) and biological installations (CAUs, ARMS, and BMUs), that were retrieved or deployed at Pearl and Hermes Atoll during cruise HA-13-05.

Site	Date	Instrument Type	Latitude	Longitude	Depth (m)	Retrieved	Deployed
PHR-33	10-Sep-13	ARMS	27.78544	-175.82355	12.8	3	—
PHR-51	10-Sep-13	ARMS	27.86679	-175.73358	13.7	—	3
PHR-52	10-Sep-13	ARMS	27.94055	-175.86165	13.1	—	3
PHR-R26	10-Sep-13	ARMS	27.78570	-175.78049	13.1	3	—
PHR-51	10-Sep-13	BMU	27.86679	-175.73358	13.7	—	5
PHR-52	10-Sep-13	BMU	27.94055	-175.86165	13.1	—	5
PHR-53	10-Sep-13	BMU	27.95493	-175.83582	14.6	—	5
PHR-33	10-Sep-13	CAU	27.78544	-175.82355	12.8	5	—

**Table C.1.1** (continued)

Site	Date	Instrument Type	Latitude	Longitude	Depth (m)	Retrieved	Deployed
PHR-51	10-Sep-13	CAU	27.86679	-175.73358	13.7	–	5
PHR-52	10-Sep-13	CAU	27.94055	-175.86165	13.1	–	5
PHR-53	10-Sep-13	CAU	27.95493	-175.83582	14.6	–	5
PHR-R26	10-Sep-13	CAU	27.78570	-175.78049	13.1	5	–
PHR-R39	10-Sep-13	CAU	27.94046	-175.86131	10.7	4	–
PHR-R44	10-Sep-13	CAU	27.91062	-175.90483	12.8	2	–
PHR-23	10-Sep-13	EAR	27.94060	-175.86173	16.5	1	–
PHR-15	10-Sep-13	STR	27.94055	-175.86164	14.3	–	1
PHR-16	10-Sep-13	STR	27.94237	-175.86342	24.1	1	1
PHR-17	10-Sep-13	STR	27.94427	-175.86496	35.7	1	–
PHR-22	10-Sep-13	STR	27.86608	-175.73118	22.6	1	–
PHR-23	10-Sep-13	STR	27.94060	-175.86173	16.5	–	1
PHR-25	10-Sep-13	STR	27.78570	-175.78048	13.1	1	–
PHR-29	10-Sep-13	STR	27.86587	-175.73026	25.3	–	1
PHR-30	10-Sep-13	STR	27.86679	-175.73357	13.7	–	1
PHR-31	10-Sep-13	STR	27.86889	-175.73489	4.9	–	1
PHR-32	10-Sep-13	STR	27.93766	-175.86350	5.8	–	1
PHR-50	11-Sep-13	ARMS	27.78237	-175.88195	14.6	–	3
PHR-54	11-Sep-13	ARMS	27.79316	-175.99722	13.7	–	3
PHR-R42	11-Sep-13	ARMS	27.75313	-175.94877	14.3	1	–
PHR-50	11-Sep-13	BMU	27.78237	-175.88195	14.6	–	5
PHR-54	11-Sep-13	BMU	27.79316	-175.99722	13.7	–	5
PHR-50	11-Sep-13	CAU	27.78237	-175.88195	14.6	–	5
PHR-54	11-Sep-13	CAU	27.79316	-175.99722	13.7	–	5
PHR-R42	11-Sep-13	CAU	27.75313	-175.94877	14.3	5	–
PHR-13	11-Sep-13	EAR	27.79101	-175.86304	11.6	1	–
PHR-19	11-Sep-13	STR	27.78196	-175.78348	22.6	1	–
PHR-2	11-Sep-13	STR	27.77473	-175.97872	1.5	1	1
PHR-26	11-Sep-13	STR	27.77275	-175.97025	9.6	1	1
PHR-27	11-Sep-13	STR	27.79316	-175.99722	13.7	–	1
PHR-28	11-Sep-13	STR	27.79578	-176.00853	24.1	–	1
PHR-9	11-Sep-13	STR	27.78173	-175.88085	38.6	1	1





**Figure C.1.2.--**Locations of deepwater CTD casts and water sampling performed at Pearl and Hermes Atoll during cruise HA-13-05. Sources: Landsat satellite imagery data from the U.S. Geological Survey; bathymetry imagery data from: ESRI, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors.

## C.2. Biological Monitoring: Microbial Sampling

Water samples for microbial analyses were collected at 5 REA sites (Table C.2.1). For more information about collections made at REA sites, see Table I.1.1 in Appendix I: “Biological Collections.”

**Table C.2.1.--**Summary of microbial water collections performed at Pearl and Hermes Atoll during cruise HA-13-05.

REA Site	Date	Reef Zone	Depth(m)	Latitude	Longitude	Microbial Samples
PHR-1004	10-Sep-13	Forereef	4.6	27.87	-175.74	1
PHR-51	10-Sep-13	Forereef	13.7	27.87	-175.73	2
PHR-52	10-Sep-13	Forereef	13.1	27.94	-175.86	2
PHR-50	11-Sep-13	Forereef	14.6	27.78	-175.88	2
PHR-54	11-Sep-13	Forereef	13.7	27.79	-176.00	3

## APPENDIX D: LISIANSKI ISLAND

The island of Lisianski is located at 26°3' N, 173°57' W in the north Pacific. For information about the methods used to perform the activities discussed in this appendix, please see Appendix A: "Methods."

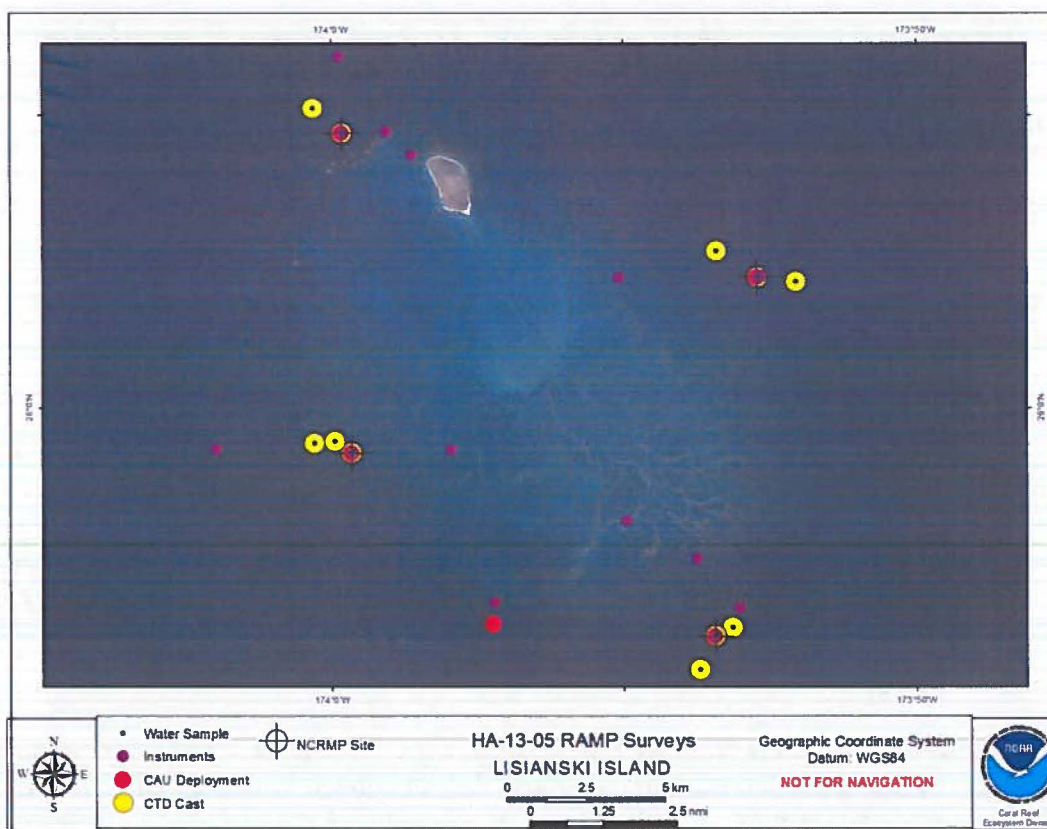
### D.1. Climate and Ocean Acidification Monitoring: Instrumentation, Biological Installations, and Water Quality

Oceanographic operations during the cruise HA-13-05 at Lisianski Island entailed numerous retrievals and deployments of oceanographic moored instruments, installation of subsurface temperature recorders (STRs), calcification acidification units (CAUs), autonomous reef monitoring structures (ARMS), sea-surface temperature (SST) buoys, wave and tide recorders (WTR), nearshore water sampling and conductivity, temperature, and depth (CTD) casts at select *NCRMP* sites, and shipboard water sampling and CTD casts offshore to a depth of 300 m, and acoustic Doppler current profiler (ADCP) transects.

Twelve shallow-water CTD casts were performed at each location where water samples were collected, this included sample locations taken in concert with the installation of *NCRMP* monitoring stations as well as at stratified random locations around the island. Seventeen shallow-water samples were collected for analysis of dissolved inorganic carbon (DIC), total alkalinity (TA), and salinity. In addition, 6 STRs were retrieved and 15 STRs were deployed. Eleven ARMS were recovered and processed for taxonomic analysis and 0 ARMS were deployed, as Lisianski Island was not considered a location for a full suite of *NCRMP* instrumentation. Seventeen CAUs were recovered and 25 CAUs were deployed at 5 locations around the island. One sea-surface temperature buoy and one WTR moorings were also retrieved (Fig. D.1.1 and Table D.1.1).

From the NOAA Ship *Hi'ialakai*, ~ 18.6 km of ADCP transect lines were run in the east and west direction, away from this island during night operations. On the reciprocal course, shipboard CTD casts were conducted to a depth of 300 m per cast every ~ 2 km for a total of 11 deep-water CTD casts around Lisianski Island. Water samples were collected concurrently at 5 depths between the surface and ~ 200 m, depending on the depth of mixed layer as determined by the CTD downcast. (Fig. D.1.2).





**Figure D.1.1.--**Mooring sites where oceanographic instruments and biological installations were retrieved or deployed and locations of nearshore CTD casts and water sampling performed at Lisianski Island during cruise HA-13-05. Sources: Landsat satellite imagery data from the U.S. Geological Survey; bathymetry imagery data from: ESRI, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors.

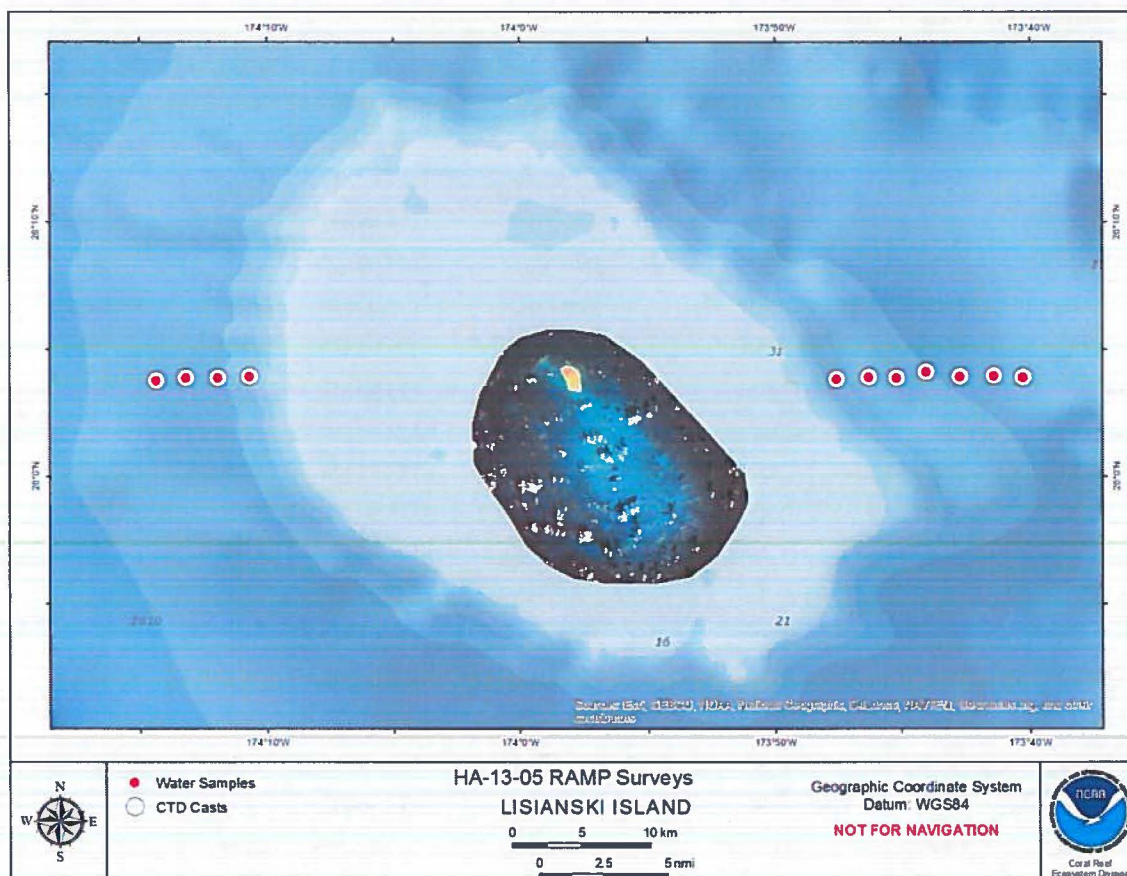
**Table D.1.1.--** Geographic coordinates and depths of the moored oceanographic instruments (STR and SST buoy) and biological installations(CAUs), that were retrieved or deployed at Lisianski Island during cruise HA-13-05.

Site	Date	Instrument Type	Latitude	Longitude	Depth (m)	Retrieved	Deployed
LIS-R14	12-Sep-13	ARMS	26.07842	-173.99699	14.3	3	—
LIS-50	12-Sep-13	CAU	26.03741	-173.87918	14.6	—	5
LIS-51	12-Sep-13	CAU	26.07842	-173.99699	14.3	—	5
LIS-9	12-Sep-13	CAU	25.95809	-173.88253	14.3	5	—
LIS-R9	12-Sep-13	CAU	26.03949	-174.01243	7.3	2	—
LIS-15	12-Sep-13	STR	26.03720	-173.91805	4.9	—	1
LIS-16	12-Sep-13	STR	26.03741	-173.87918	16.2	—	1
LIS-17	12-Sep-13	STR	26.03765	-173.87764	25.3	—	1
LIS-18	12-Sep-13	STR	26.07193	-173.97743	0.9	—	1



**Table D.1.1** (continued)

Site	Date	Instrument Type	Latitude	Longitude	Depth (m)	Retrieved	Deployed
LIS-19	12-Sep-13	STR	26.07869	-173.98471	4.3	–	1
LIS-4	12-Sep-13	STR	26.10018	-173.99803	24.1	–	1
LIS-9	12-Sep-13	STR	26.07842	-173.99699	14.3	1	1
LIS-18	13-Sep-13	ARMS	26.04280	-173.99403	0.9	5	–
LIS-R10	13-Sep-13	ARMS	25.94455	-173.95357	13.1	3	–
LIS-18	13-Sep-13	CAU	26.04280	-173.99403	0.9	5	–
LIS-52	13-Sep-13	CAU	25.93497	-173.89110	16.8	–	5
LIS-53	13-Sep-13	CAU	25.93831	-173.95396	14.9	–	5
LIS-54	13-Sep-13	CAU	25.98702	-173.99438	11.3	–	5
LIS-R10	13-Sep-13	CAU	25.94455	-173.95357	13.1	5	–
LIS-1	13-Sep-13	SST	25.96776	-173.91595	0.3	1	–
LIS-1	13-Sep-13	STR	25.96776	-173.91595	0.3	1	1
LIS-10	13-Sep-13	STR	25.93465	-173.89123	26.2	–	1
LIS-11	13-Sep-13	STR	25.93497	-173.89111	17.4	–	1
LIS-12	13-Sep-13	STR	25.95710	-173.89615	5.5	–	1
LIS-13	13-Sep-13	STR	25.98791	-173.96602	4.9	–	1
LIS-14	13-Sep-13	STR	25.98809	-174.03298	25.0	–	1
LIS-5	13-Sep-13	STR	25.96776	-173.91595	9.8	1	1
LIS-6	13-Sep-13	STR	25.98700	-173.99441	14.5	1	1
LIS-7	13-Sep-13	STR	25.94303	-173.88421	21.9	1	–
LIS-8	13-Sep-13	STR	25.94455	-173.95357	12.8	1	–
LIS-7	13-Sep-13	WTR	25.94303	-173.88421	21.9	1	–



**Figure D.1.2.--**Locations of deep-water CTD casts and water sampling performed at Lisianski Island during cruise HA-13-05. Sources: Landsat satellite imagery data from the U.S. Geological Survey; bathymetry imagery data from: ESRI, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors.

## D.2. Biological Monitoring: Microbial Sampling

Water samples for microbial analyses were collected at 4 REA sites (Table D.2.1). For more information about collections made at REA sites, see Table I.1.1 in Appendix I: “Biological Collections.”

**Table D.2.1.--**Summary of microbial water collections performed at Lisianski Island during cruise HA-13-05.

REA Site	Date	Reef Zone	Depth(m)	Latitude	Longitude	Microbial Samples
LIS-50	12-Sep-13	Forereef	14.6	26.03741	-173.87918	2
LIS-51	12-Sep-13	Forereef	14.3	26.07842	-173.99699	2
LIS-52	13-Sep-13	Forereef	16.8	25.93497	-173.89110	2
LIS-54	13-Sep-13	Forereef	11.3	25.98702	-173.99438	5





## APPENDIX E: OAHU ISLAND

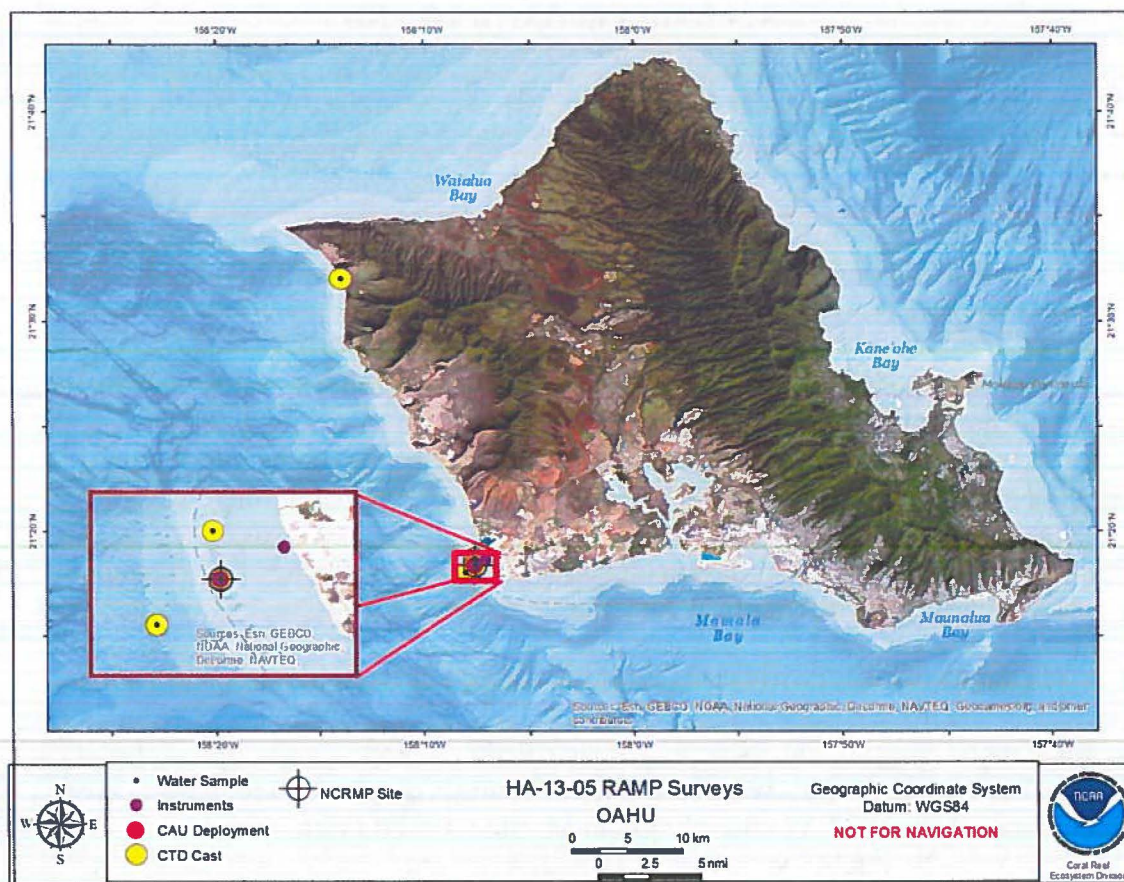
The island of Oahu is located at 21°28' N, 157°59' W in the north Pacific and is part of the main Hawaiian Island chain. For information about the methods used to perform the activities discussed in this appendix, please see Appendix A: "Methods."

### **E.1. Climate and Ocean Acidification Monitoring: Instrumentation, Biological Installations and Water Quality**

Oceanographic operations during the cruise HA-13-05 at Oahu Island entailed numerous retrievals and deployments of oceanographic moored instruments, installation of subsurface temperature recorders (STRs), calcification acidification units (CAUs), autonomous reef monitoring structures (ARMS), bioerosion monitoring units (BMU), nearshore water sampling and conductivity, temperature, and depth (CTD) casts at select *NCRMP* sites, and shipboard water sampling and CTD casts offshore to a depth of 300 m, and acoustic Doppler current profiler (ADCP) transects.

Four shallow-water CTD casts were performed at each location where water samples were collected, this included sample locations taken in concert with the installation of *NCRMP* monitoring stations as well as at stratified random locations around the island. Five shallow-water samples were collected for analysis of dissolved inorganic carbon (DIC), total alkalinity (TA), and salinity. In addition, 0 STRs were retrieved and 3 STRs were deployed. No ARMS were recovered and processed for taxonomic analysis and 3 ARMS were deployed. No CAUs were recovered and 5 CAUs were deployed at 1 location around the island. Five BMUs were collocated at the CAU sites as part of the *NCRMP* survey sites. (Fig. E.1.1 and Table E.1.1).

From the NOAA Ship *Hi'ialakai*, ~ 9.8 km of ADCP transect lines were run in the east and west direction, away from this island during night operations. On the reciprocal course, shipboard CTD casts were conducted to a depth of 300 m per cast every ~ 2 km for a total of 6 deep-water CTD casts around Lisianski Island. Water samples were collected concurrently at 5 depths between the surface and ~ 200 m, depending on the depth of mixed layer as determined by the CTD downcast. (Fig. D.1.2).

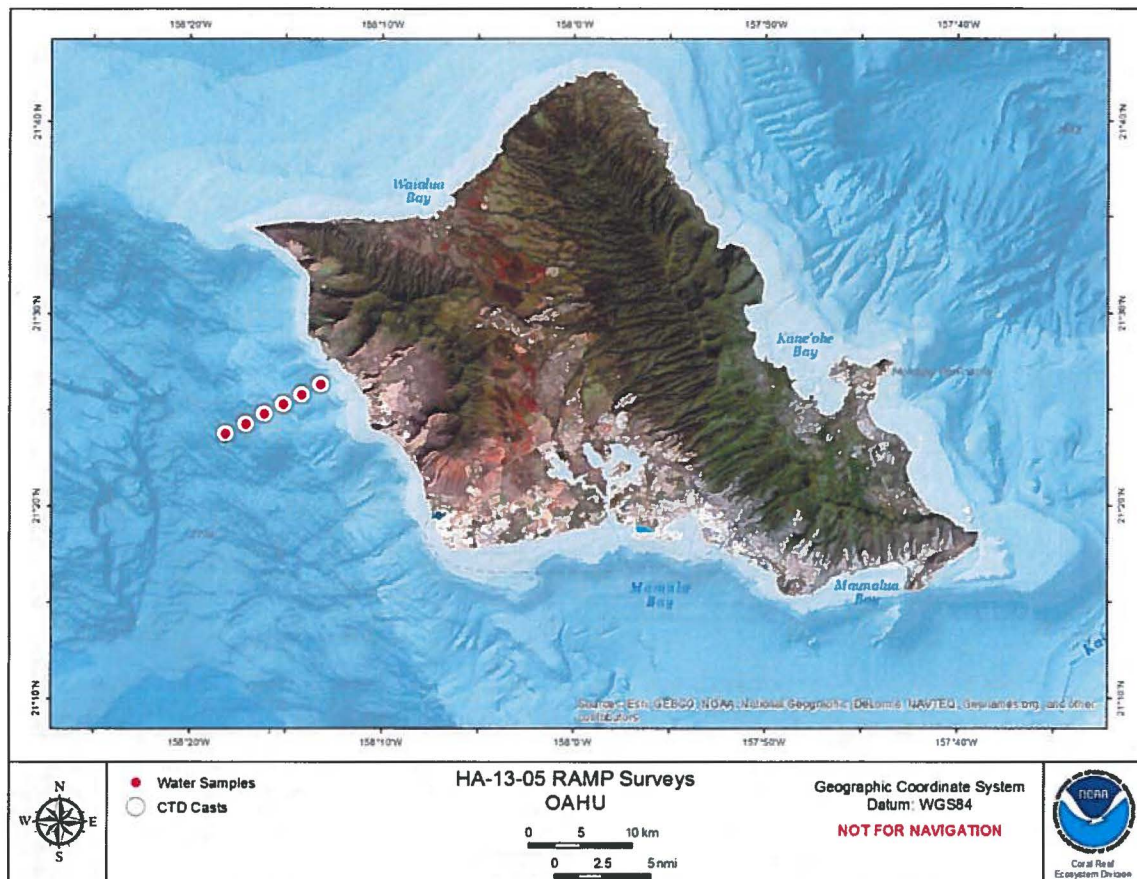


**Figure E.1.1.--**Mooring sites where oceanographic instruments and biological installations were retrieved or deployed and locations of nearshore CTD casts and water sampling performed at Oahu Island during cruise HA-13-05. Sources: Landsat satellite imagery data from the U.S. Geological Survey; bathymetry imagery data from: ESRI, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors.

**Table E.1.1.--**Geographic coordinates and depths of the moored oceanographic instruments (STR) and biological installations (CAUs and ARMS), that were retrieved or deployed at Oahu Island during cruise HA-13-05.

Site	Date	Instrument Type	Latitude	Longitude	Depth (m)	Retrieved	Deployed
OAH-019	18-Sep-13	STR	21.30949	-158.11841	5.2	—	1
OAH-020	18-Sep-13	STR	21.30573	-158.12658	26.8	—	1
OAH-21	18-Sep-13	STR	21.30566	-158.12623	15.2	—	1
OAH-21	18-Sep-13	CAU	21.30566	-158.12622	15.2	—	5
OAH-21	18-Sep-13	BMU	21.30566	-158.12622	15.2	—	5
OAH-21	18-Sep-13	ARMS	21.30566	-158.12622	15.2	—	3

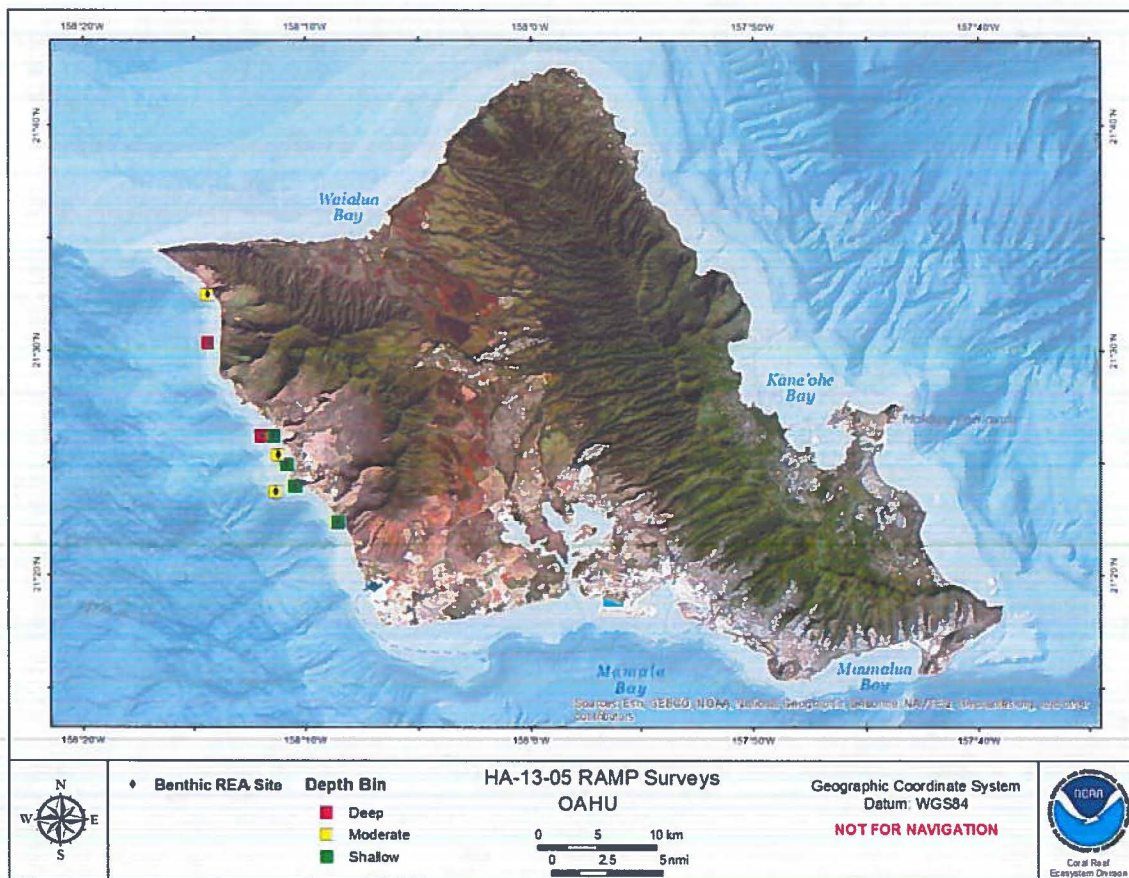




**Figure E.1.2.--**Locations of deepwater CTD casts and water sampling performed at Oahu Island during cruise HA-13-05. Sources: Landsat satellite imagery data from the U.S. Geological Survey; bathymetry imagery data from: ESRI, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors.

## E.2. Biological Monitoring: Benthic Surveys and Microbial Sampling

REA benthic sampling utilized a two-stage stratified random sampling design approach that incorporated both broad categories of reef structure and depth to define habitat strata. Belt-transect surveys were conducted and photographs were taken along transect lines at 9 sites randomly located on Oahu Island. 0 microbial water samples were collected on this day at the benthic REA survey sites. (Figure E.2.1 and Table E.2.1). For more information about collections made at REA sites, see Table I.1.1 in Appendix I: “Biological Collections.”



**Figure E.2.1.--**Locations of REA benthic sites surveyed at Oahu Island during cruise HA-13-05. All of these REA sites were selected using a stratified random design. Sources: Landsat satellite imagery data from the U.S. Geological Survey; bathymetry imagery data from: ESRI, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors.

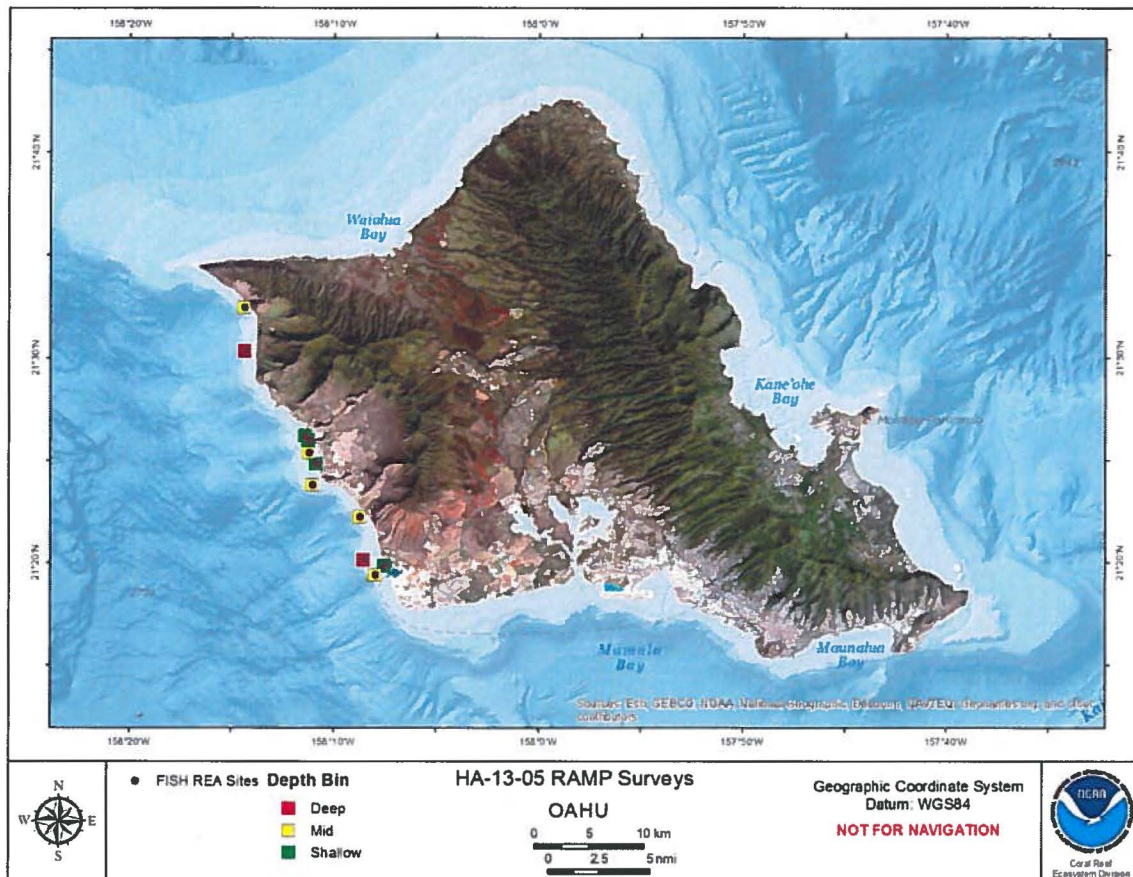
**Table E.2.1.--**Summary of REA benthic surveys and microbial water samples performed at Oahu Island during cruise HA-13-05.

REA Site	Date	Depth Bin	Reef Zone	Depth(m)	Latitude	Longitude
OAH-1562	18-Sep-13	Deep	Forereef	20.42	21.43638	-158.2
OAH-1572	18-Sep-13	Shallow	Forereef	5.5	21.37272	-158.142
OAH-1585	18-Sep-13	Moderate	Forereef	7.6	21.42258	-158.187
OAH-1586	18-Sep-13	Shallow	Forereef	5.8	21.43641	-158.19
OAH-1587	18-Sep-13	Moderate	Forereef	16.2	21.39527	-158.189
OAH-1604	18-Sep-13	Shallow	Forereef	4.3	21.41518	-158.181
OAH-1629	18-Sep-13	Moderate	Forereef	12.2	21.54117	-158.24
OAH-1910	18-Sep-13	Deep	Forereef	22.3	21.50546	-158.239
OAH-1999	18-Sep-13	Shallow	Forereef	3.7	21.3993	-158.175



### E.3. Biological Monitoring: Reef Fish Community

REA fish survey sites were chosen using a stratified random design. Stationary-point-count surveys were conducted at 11 REA sites at Oahu Island over three different habitat strata: deep, moderate, and shallow forereef (Fig.E.3.1 and Table E.3.1). No fishes were collected during these surveys.



**Figure E.3.1.--**Locations of REA fish sites surveyed at Oahu Island during cruise HA-13-05. All of these REA sites were selected using a stratified random design. Sources: Landsat satellite imagery data from the U.S. Geological Survey; bathymetry imagery data from: ESRI, GEBCO, NOAA, National Geographic, DeLorme, NAVTEQ, Geonames.org, and other contributors.



**Table E.3.1.--Summary of sites where REA fish surveys were conducted at Oahu Island during cruise HA-13-05.**

REA_Site	Date	DepthBin	Reef_Zone	Depth_(m)	Latitude	Longitude
OAH-569	18-Sep-13	Deep	Forereef	27	21.34	-158.14
OAH-572	18-Sep-13	Moderate	Forereef	13	21.37	-158.15
OAH-578	18-Sep-13	Moderate	Forereef	12	21.32	-158.13
OAH-585	18-Sep-13	Moderate	Forereef	7	21.42	-158.19
OAH-586	18-Sep-13	Shallow	Forereef	6	21.44	-158.19
OAH-587	18-Sep-13	Moderate	Forereef	13	21.40	-158.18
OAH-604	18-Sep-13	Shallow	Forereef	5.5	21.41	-158.18
OAH-605	18-Sep-13	Shallow	Forereef	5	21.33	-158.13
OAH-629	18-Sep-13	Moderate	Forereef	12.1	21.54	-158.24
OAH-630	18-Sep-13	Shallow	Forereef	5.8	21.43	-158.19
OAH-910	18-Sep-13	Deep	Forereef	22.3	21.51	-158.24

## APPENDIX F: BIOLOGICAL COLLECTIONS

Biological and other samples were collected at Hawai`i Island, Kaua`i Island, Lāna`i Island, Maui Island, Moloka`i Island, Ni`ihau Island, and O`ahu Island and their surrounding waters for multiple research purposes. These collections are listed here in Table F.1.1.

**Table F.1.1.--**Samples collected at French Frigate Shoals, Pearl and Hermes Atoll, and Lisianski Island for various analyses during cruise HA-13-05.

REA Site	Date	Latitude	Longitude	Specimen Collected	Number of Samples	Depth (m)
Microbial Collections: Water Samples, Coral Rubble, and Macroalgae						
FFS-1006	06-Sep-13	23.79234	-166.25368	2 L	2	13.7
FFS-1006	06-Sep-13	23.79234	-166.25368	20 L	3	13.7
FFS-40	06-Sep-13	23.63686	-166.18515	2 L	2	13.7
FFS-42	07-Sep-13	23.87806	-166.291	2 L	2	13.7
FFS-42	07-Sep-13	23.87806	-166.291	20 L	3	13.7
FFS-43	07-Sep-13	23.88021	-166.27707	2 L	2	13.7
FFS-44	07-Sep-13	23.83515	-166.11682	2 L	2	13.7
LIS-50	12-Sep-13	26.03741	-173.87918	2 L	2	13.7
LIS-51	12-Sep-13	26.07842	-173.99699	2 L	2	13.7
LIS-52	13-Sep-13	25.93497	-173.8911	2 L	2	9.8
LIS-54	13-Sep-13	25.98702	-173.99438	2 L	2	9.8
LIS-54	13-Sep-13	25.98702	-173.99438	20 L	3	9.8
LIS-54	13-Sep-13	25.98702	-173.99438	Coral rubble	1	9.8
LIS-54	13-Sep-13	25.98702	-173.99438	Unidentified macroalgae	1	9.8
PHR-1004	10-Sep-13	27.86674	-175.73599	20 L	3	4.6
PHR-50	11-Sep-13	27.78236842	-175.88195	2 L	2	15.2
PHR-51	10-Sep-13	27.86679	-175.73358	2 L	2	12.8
PHR-52	10-Sep-13	27.94055	-175.86165	2 L	2	13.1
PHR-54	11-Sep-13	27.79316	-175.99722	2 L	2	12.8
PHR-54	11-Sep-13	27.79316	-175.99722	20 L	3	12.8
Algal Collections: Ocean Acidification						
FFS-12	05-Sep-13	23.63835	-166.18005	CAU unit	5	11.6
FFS-21	06-Sep-13	23.84695	-166.32695	CAU unit	5	12.2
FFS-34	05-Sep-13	23.62792	-166.13538	CAU unit	5	9.1
FFS-H6	07-Sep-13	23.88043	-166.27307	CAU unit	5	7.6
FFS-R46	06-Sep-13	23.76928	-166.26173	CAU unit	5	8.2
PHR-33	10-Sep-13	27.78544	-175.82355	CAU unit	5	12.8
PHR-R26	10-Sep-13	27.7857	-175.78049	CAU unit	5	14.3

**Table F.1.1 (continued)**

REA Site	Date	Latitude	Longitude	Specimen Collected	Number of Samples	Depth (m)
PHR-R39	10-Sep-13	27.94046	-175.86131	CAU unit	4	12.8
PHR-R42	11-Sep-13	27.75313	-175.94877	CAU unit	4	13.7
PHR-R44	10-Sep-13	27.91062	-175.90483	CAU unit	2	14.6

Invertebrate Collections: Bivalve Sand Collection						
FFS-21	06-Sep-13	23.84695	-166.32695	1 Liter	3	12.2
FFS-43	07-Sep-13	23.88021	-166.27707	1 Liter	3	16.8
FFS-H6	07-Sep-13	23.88043	-166.27307	1 Liter	2	7.6
FFS-R46	06-Sep-13	23.76928	-166.26173	1 Liter	3	8.2
LIS-R10	13-Sep-13	25.94455	-173.95357	1 Liter	3	15.2
LIS-R14	12-Sep-13	26.07842	-173.99699	1 Liter	3	20.4
LIS-R9	12-Sep-13	26.03949	-174.01243	1 Liter	3	14.9
PHR-50	11-Sep-13	27.78237	-175.88195	1 Liter	3	14.6
PHR-54	11-Sep-13	27.79316	-175.99722	1 Liter	2	13.7
PHR-R26	10-Sep-13	27.7857	-175.78049	1 Liter	3	14.3
PHR-R39	10-Sep-13	27.94046	-175.86131	1 Liter	3	12.8
PHR-R44	10-Sep-13	27.91062	-175.90483	1 Liter	3	14.6