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# CRUISE REPORT[1]

**VESSEL:** NOAA Ship *Hi`ialakai*, Cruise HA-14-01 Leg I

**CRUISE PERIOD:** 5- 24 March, 2014

**AREA OF OPERATION:** Wake Atoll

**TYPE OF OPERATION:** Personnel from the Coral Reef Ecosystem Division (CRED) of the NOAA Pacific Islands Fisheries Science Center, the NOAA Pacific Islands Regional Office, and San Diego State University, conducted interdisciplinary surveys of benthos, fishes, and climate in waters surrounding Wake Atoll as part of the National Coral Reef Monitoring Plan (NCRMP). All activities described in this report were covered by the following permits: U.S. Army Corps of Engineers Permit: USACE POH-2009-00083 (effective date: March 18, 2014, expiration date March 18, 2017); U.S. Fish and Wildlife Service, National Wildlife Refuge System, Research and Monitoring Special Use Permit: 12518-1404; U.S. National Park Service, Scientific Research and Collection Permit: WAPA-2014SCI-002 (effective March 21, 2014; expiration March 31, 2014).

## ITINERARY:

Note: This report includes all monitoring activities completed around Wake Atoll during HA-14-01 Leg I. All work conducted around the island of Guam during HA-14-01 Leg I are featured in a separate report along with all the monitoring activities completed during HA-14-01 Legs II and III.

Daily field operations included Rapid Ecological Assessment (REA) benthic surveys, REA fish surveys, towed-diver surveys, near-shore conductivity, temperature, and depth (CTD) casts; water samples for dissolved inorganic carbon (DIC), total alkalinity (TA),

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[1] PIFSC Cruise Report CR-15-003
Issued 22 April 2015

and salinity, and water samples for microbial analyses. Unless otherwise specified in the following daily summaries, these surveys occurred during each operational day.

| | |
|---|---|
| 5 March | Start of cruise; embarked all scientific personnel. The departure of the mission was delayed by approximately 5 hours. After the previously scheduled compass swing for calibration purposes, it was necessary to transport the compass technician to shore via small boat. An additional delay was accrued to wait for a boat sponson part needed for a ship-board small boat. |
| 6 March | Transit day. |
| 7 March | Transit day. |
| 8 March | Transit day. |
| 9 March | Transit day. |
| 11 March | Transit day — Crossed dateline: March 10 became March 11. |
| 12 March | Transit day. |
| 13 March | Transit day. |
| 14 March | Arrived at Wake Island at 6:50 am. The weather conditions were poor even on the south side of the island (leeward). The winds ranged from 24to 32 knots with higher gusts throughout the day. The sea state was choppy at best and consistent of ground swell and wind chop. After periodic assessments of the sea state and wind conditions throughout the morning as well as operational safety (e.g., the ship's ability to launch and retrieve the small boats safely, coxswain's ability to maintain visual contact with the divers' bubbles throughout their dive), all dive operations for the day were called off. |
| 15 March | No operations conducted due to inclement weather. |
| 16 March | Conducted operations along S Wake Atoll; Retrieved 2 STRs and one ARMs; Deployed 4 STRs and 6 ARMs. |
| 17 March | Conducted operations at Wake Atoll; Retrieved 3 ARMs, 6 CAUs, and 2 STRs; Deployed 10 CAUs, 10 BMUs, and 2 STR. |
| 18 March | Conducted operations along the North and East side of Wake Atoll; Retrieved 3 STRs; Deployed 6 STRs, 6 ARMs, 10 CAUs and 10 BMUs. |

## Biological Monitoring

### REA 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 health 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

### REA Fish Surveys:

- 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

### Towed-diver Surveys:

- Digital still photographs and video of benthic habitats
- Counts of target macro invertebrates, including crown-of-thorns seastars, sea cucumbers, sea urchins, and giant clams
- Quantitative assessments of large ($\geq$ 50 cm in total length) reef fishes to species level
- Quantitative and qualitative assessments of key protected species and species of concern, including cetaceans, sea turtles, and rare fishes
- Benthic habitat characterization, including visual estimates of habitat complexity, habitat type, and cover of corals, stressed corals, macroalgae, and crustose coralline red algae
- Temperature and depth data

## Climate and Ocean Acidification Monitoring

### Oceanographic Instrumentation and Biological Installations:

- Seawater temperature at 1, 5, 15, 25 m depths
- Assessment of taxonomic diversity of coral reef species by collection of invertebrate specimens from retrieved ARMS
- Installation of CAUs to allow for future assessment of $CaCO_3$ deposition rates once they are retrieved in about 3 years
- Installation of BMUs to allow for future assessment of bioerosion rates once they are retrieved in about 3 years

### Nearshore Oceanography from Small Boats:

- Shallow-water CTD profiles to depths ≤ 30 m, including all sites where CAUs were installed and selected benthic REA sites
- Water samples for salinity, DIC, and TA collected in concert with shallow-water (≤ 30 m) CTD casts

**SCIENTIFIC PERSONNEL:**

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Submitted by: _____
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Submitted by: _____
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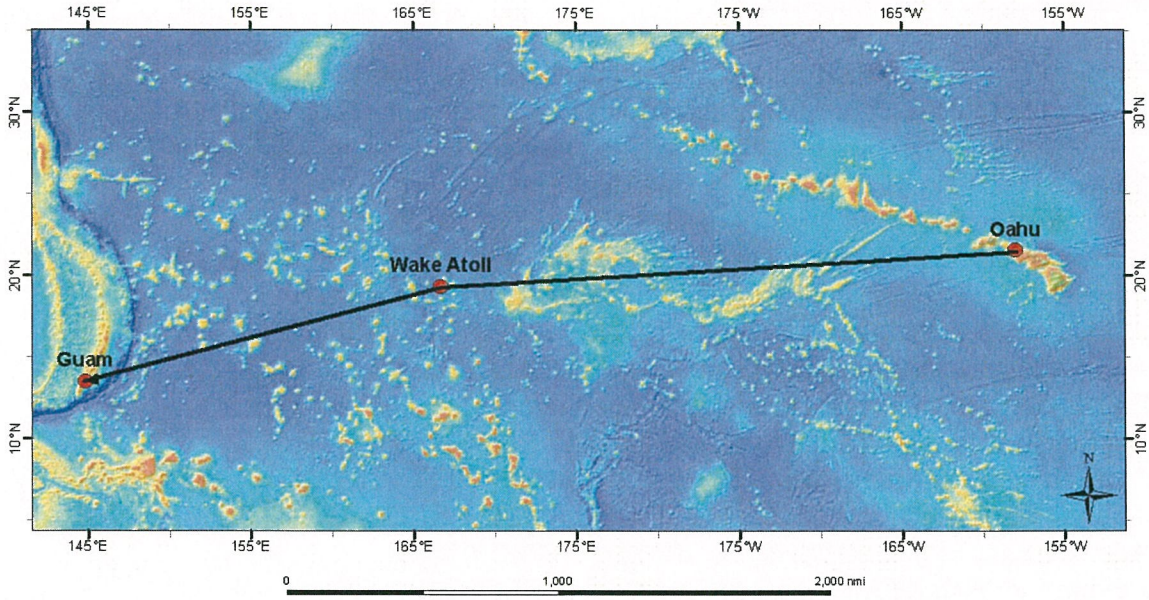


**Figure 1.**--Track of the NOAA Ship *Hi`ialakai* for the cruise HA-14-01, March 5 - 24, 2014, with Wake Atoll surveyed. Satellite image: SIO, NOAA, U.S. Navy, NGA, GEBCO (Becker, 2009; Smith and Sandwell, 1997) © 2008 The Regents of the University of California.

# 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-14-01 on the NOAA Ship *Hi`ialakai* during the period of March 5-24, 2014. The first Pacific RAMP expedition at Wake Atoll was conducted in 2005.

## Climate and Ocean Acidification Monitoring

### A.1. Instrumentation, Biological Installations, and Water Quality

*(Jamison Gove, James Morioka, Russell Reardon, Max Sudnovsky, Molly Timmers, and Charles Young)*

Four main activities were conducted by the instrumentation, biological installations, and water quality team: (1) near-shore oceanographic and water-quality surveys; (2) deployment and retrieval of an array of subsurface moored instrumentation and instrumentation 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.

Climate and ocean acidification monitoring efforts at each survey site fall into four different levels of increasing scientific investigation. These are intended to document the island-scale, water chemistry, spatial and temporal variability of reef water thermal structure across a depth gradient, and the integrated biological responses of the reef community to the prevailing chemical and physical conditions. Most of the CRED's efforts focus on establishing Class 0 and Class 2 sites at select locations distributed along the four cardinal directions around each island surveyed.

1- Class 0 sites: Only discrete water samples are collected and analyzed for dissolved inorganic carbon (DIC) and total alkalinity (TA).

2- Class 1 sites: Only SBE-56 subsurface temperature recorders (STRs) are deployed.

3- Class 2 sites: Include collection of discrete water for DIC and TA, and STR deployments, in addition to benthic surveys and photo quadrat records. Biological installation, including Calcification Accretion Units (CAUs),

Bioerosion Monitoring Units (BMUs), Autonomous Reef Monitoring Structures (ARMS), and coral coring are added.

4- Class 3 sites: A MAPpCO2 buoy system is added to the Class 2 site setup.

For "class 2 sites" and above, thermal structure measurements are obtained based on the deployment of temperature sensors (Seabird Electronics, SBE-56) along a fore-reef transect at 1 m, 5m, 15 m, and 25 m depth; each SBE-56 records the near-reef water temperature at the same time, on a 5-minute interval, for the duration of the instrument's deployment. Within this context, a permanent water-quality, temperature, and biological survey/sampling site, designated as *NCRMP Monitoring Station*, is established at the 15-m depth STR location, at select areas. In addition to the SBE-56 the NCRMP Monitoring Station includes: deployment of 3 ARMS units, 5 CAUs, 5 BMUs, and 1 STR; collection of 3 carbonate chemistry water samples (with associated CTD casts), acquisition of photo quadrat 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 and biological instruments were retrieved or deployed during this cruise.

**Subsurface Temperature Recorder (STR):** provides high-resolution temperature data (SBE-39 or SBE-56). Data are internally recorded at 30-min intervals. This type of subsurface instrument is deployed at depths of 0.5–40 m.

**Wave-and-tide Recorder (WTR):** Provides high-resolution wave and tide records (SBE 26plus Sea gauge recorder, accuracy of 0.01% in pressure). Data are internally recorded and sample intervals vary depending on duration of deployment. This type of subsurface instrument typically is deployed at depths of 10–25 m.

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

**Bioerosion Monitoring Unit (BMU):** provides 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 Casts:** a CTD profiler deployed from a small boat provided water column data on temperature, conductivity (which is related to salinity) and pressure, which is related to depth (SBE 19plus Seacat Profiler). A transmissometer (C-Star, WET Labs, Philomath, OR) provided profiles of beam transmittance, which is related to turbidity. A dissolved oxygen sensor (SBE 43, accuracy of 2% of saturation) also was attached and measurements were made in concert with CTD measurements. 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 s-1 to depths up to 30 m.

**Deepwater (Shipboard) CTD Casts**: a ship-based CTD profiler provided high-resolution conductivity, temperature, and pressure data (SBE 911plusCTD, accuracy of 0.003 S m-1 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-1 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 casts.

## A.2. Benthic Surveys and Microbial Sampling

*(Hatsue Bailey, Marie Ferguson, Joao Garriques, Emma George, Kevin O'Brien, Dione Swanson)*

A two-stage stratified random sampling design was employed to survey the Rapid Ecological Assessment (REA) sites on Wake Atoll. The survey domain encompassed 99.6% of the mapped area of reef and hard bottom fore reef habitats and was divided into strata based on three depth categories: shallow (0–6 m), mid (> 6–18 m) and deep

(> 18–30 m). Allocation of sampling effort was proportional to strata area. Sites were randomly selected within each stratum.

### A.2.1. Benthic composition and coral demography

Surveys at each site were conducted within two 10 $m^2$ belt transects. Adult coral colonies (≥ 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 (a total of 3 $m^2$ per transect): 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.

Photo quadrat images were collected to record the benthos at predetermined points along the same 2 transect lines with a high-resolution digital camera mounted on a photo quadrat 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).

In addition to site-specific REA surveys, broad-scale towed-diver surveys were used to determine the benthic composition of shallow-water habitats around each island and to quantify the abundance of target macro invertebrates, including crown-of-thorns seastars (COTS), sea urchins, sea cucumbers, and giant clams. A pair of divers, by means similar to a manta-tow technique, were towed 60 m behind a small boat, a 6-m survey launch from SAFE Boats International (Port Orchard, WA), with one diver quantifying the benthos and the other quantifying fish populations. Each towed-diver survey lasted 50 min, broken into 10 segments of 5 min each, and covered ~ 2 km. To georeference the survey launch's track, latitude and longitude coordinates were recorded at 5-s intervals using a Garmin GPSMap 76 global positioning system (GPS) unit on the boat. A custom algorithm was used to calculate the track of the divers based on speed and course of the boat and depth of the diver. Each towed-diver platform, or tow board, was equipped with

an SBE 39 temperature and depth recorder programmed to record at 5-s intervals. At the end of each day, data were downloaded, processed, and presented in ArcGIS and can be displayed in conjunction with IKONOS satellite imagery, NOAA chart data, or other spatial data layers.

Towed-diver benthic surveys recorded habitat type and complexity; percentages of cover of benthic fauna, including hard corals, stressed hard corals, octocorals, macroalgae, and crustose coralline red algae, and of physical features, including sand and rubble; and counts of target macro invertebrates and marine debris. Towed divers classified percentage of cover using a system of 10 bins, ranging from 0% to 100% cover of the benthos. Target macro invertebrates were counted up to 25 individuals per segment and then binned into larger groups when exceeding 25. The benthic tow board was equipped with a downward-facing, high-resolution digital still camera. The camera took a photograph of the substrate every 15 s. These photos, like the SBE 39 data, are linked spatially with GPS track files taken aboard the survey launch. Benthic photos can be analyzed later for community structure information.

### A.2.2. Microbial Communities and Water Chemistry

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 ($NO_2$; $NO_3$; ammonium, $NH_4$; and $PO_4^{3-}$), 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 ($NO_x$+$NH_4$) 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 obtained at select REA sites (locations with supporting fish or benthic data).

The following data items were collected 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-μm filters, stained using SYBR Gold, Molecular Probes Inc., Eugene, Ore.)

- Microbial (Bacteria and Archaea) size structure : 2 replicates (0.2-μm 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-μm 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 μm)
- 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-μm 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-μm 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° 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 chlorophyll-*a*.

Six 1-mL samples of water from each REA site were pushed through a 20-μm filter. This filtrate was dispensed into cryovials (6 × 1 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°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°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 μm 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-μm 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 ~ 500 mL. This concentrate was then filtered through 0.45-μm filters to capture microbes (Bacteria and Archaea). These filters were frozen at – 20°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-μm filter, and the sample was stored at 4°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°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. Surveys of Reef Fishes

*(Jacob Asher, Paula Ayotte, Edmund Coccagna, Kelvin Gorospe, Andrew Gray, Adel Heenan, Kevin Lino)*

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 fore-reef, 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.

In addition to site-specific REA surveys, broad-scale towed-diver surveys were used to characterize the fish communities of shallow-water habitats around each island. A pair of divers, by means similar to a manta-tow technique, was towed 60 m behind a small boat, a 6-m survey launch from SAFE Boats International, with one diver quantifying fish populations and the other quantifying the benthos. Each towed-diver survey lasted 50 min, broken into 10 segments of 5-min each, and covered ~ 2 km. To georeference the survey launch's track, latitude and longitude coordinates were recorded at 5-s intervals using a Garmin GPSMap 76 GPS unit on the boat. A custom algorithm was used to calculate the track of the divers based on the track, speed, and course of the boat and depth of the diver. Each towed-diver platform, or tow board, was equipped with an SBE 39 temperature and depth recorder set to record at 5-s intervals. At the end of each day, data were downloaded, processed, and presented in ArcGIS and can be displayed in

conjunction with IKONOS satellite imagery, NOAA chart data, or other spatial data layers.

Towed-diver fish surveys record, to the lowest possible taxon, all fishes > 50 cm in total length along a 10-m swath during each 5-min segment. Individual fishes were counted and their species (or lowest possible taxon) and length in centimeters recorded. Sightings of species of particular concern observed outside the survey swath were classified as presence/absence data and were recorded separately from the quantitative swath data. At the end of each day, data were transcribed from field data sheets into a centralized Microsoft Access database. Biomass values are calculated using species-specific length-weight parameters and are normalized by area (i.e., kg 100 $m^{-2}$). The fish tow board was equipped with a forward-looking digital video camera that created a visual archive of the survey track that can be used to evaluate stochastic changes in reef environments, particularly following episodic events, such as coral bleaching or grounding of a vessel.

# APPENDIX B: WAKE ATOLL

Wake Atoll is located at 19°36′ N, 166°30′ E in the North Pacific and is part of the Pacific Remote Islands Marine National Monument. For information about the methods used to perform the activities discussed in this appendix, please see Appendix A: "Methods."

## B.1. Instrumentation, Biological Installations, and Water Quality

Oceanographic operations during the cruise HA-14-01 at Wake 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 (BMU), near-shore water sampling and conductivity, temperature, and depth (CTD) casts at select *NCRMP* sites.

Sixteen 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. Nineteen shallow-water samples were collected for analysis of dissolved inorganic carbon (DIC), total alkalinity (TA), and salinity. In addition, 11 STRs were retrieved and 14 STRs were deployed. Seven ARMS were recovered and processed for taxonomic analysis and 12 ARMS were deployed. Twenty-five CAUs were deployed at 5 locations around the island, 20 BMUs were collocated at the CAU sites which were directly a part of the NCRMP monitoring stations, and 24 CAUs were retrieved (Fig. B.1.1and Table B.1.1).

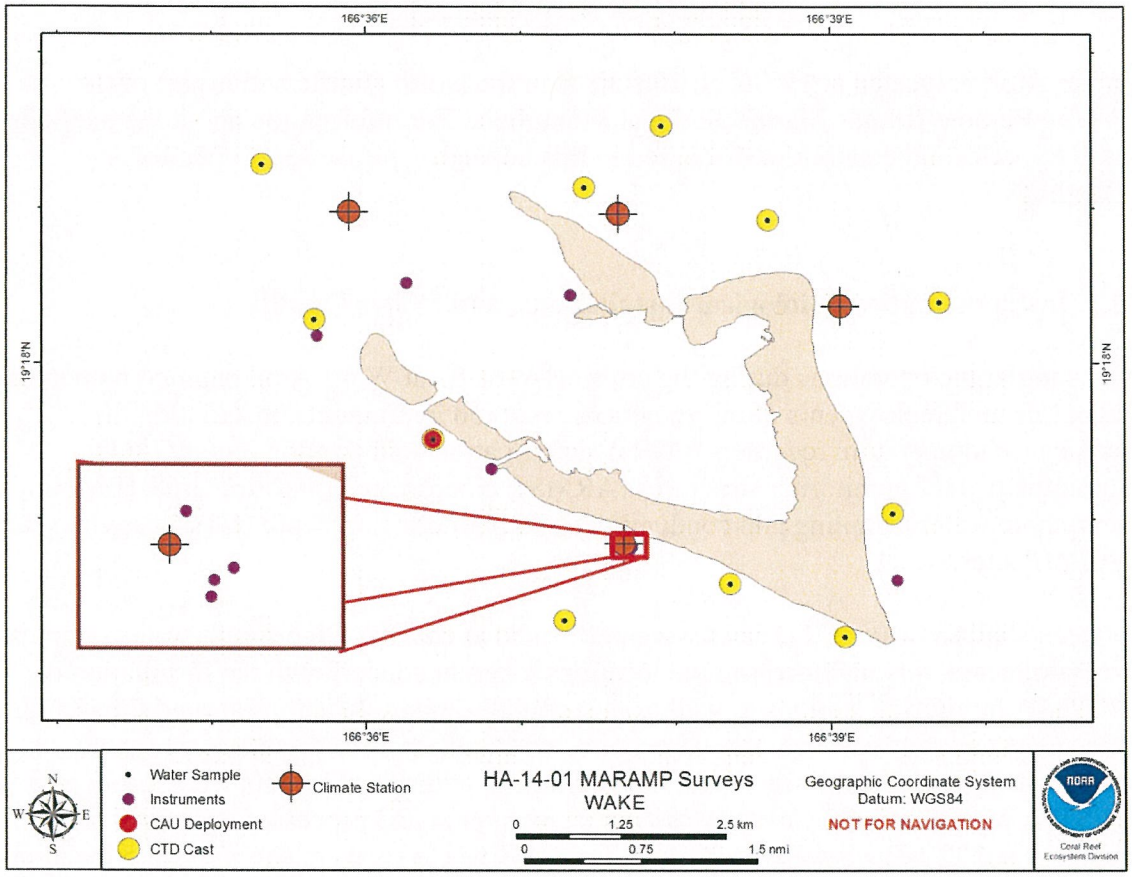


**Figure B.1.1.--**Mooring sites where oceanographic instruments and biological installations were retrieved or deployed, and locations of near-shore CTD casts and water sampling performed at Wake Atoll during cruise HA-14-01.

**Table B.1.1.--** Geographic coordinates and depths of the moored oceanographic instruments (STR and EAR) and biological installations(CAU, ARMS, and BMUs), that were retrieved or deployed at Wake Atoll during cruise HA-14-01.

| Site | Date | Instrument Type | Latitude | Longitude | Depth (m) | Retrieved | Deployed |
|---|---|---|---|---|---|---|---|
| WAK_OCEAN_001 | 15-Mar-14 | STR | 19.28064 | 166.62777 | 13.7 | 0 | 1 |
| WAK_OCEAN_006 | 15-Mar-14 | STR | 19.31627 | 166.59833 | 11.9 | 1 | 0 |
| WAK_OCEAN_019 | 15-Mar-14 | STR | 19.28017 | 166.62840 | 19.2 | 1 | 0 |
| WAK_OCEAN_020 | 15-Mar-14 | STR | 19.28064 | 166.62777 | 15.5 | 0 | 1 |
| WAK_OCEAN_030 | 15-Mar-14 | STR | 19.27994 | 166.62836 | 25.3 | 0 | 1 |
| WAK_OCEAN_033 | 15-Mar-14 | STR | 19.28111 | 166.62801 | 5.2 | 0 | 1 |
| WAK-20 | 16-Mar-14 | ARMS | 19.28065 | 166.62778 | 16.5 | 0 | 3 |
| WAK-20 | 16-Mar-14 | BMU | 19.28065 | 166.62778 | 14.6 | 0 | 5 |
| WAK-20 | 16-Mar-14 | CAU | 19.28065 | 166.62778 | 14.6 | 5 | 5 |
| WAK_OCEAN_007 | 16-Mar-14 | STR | 19.31632 | 166.59797 | 24.7 | 1 | 1 |
| WAK_OCEAN_018 | 16-Mar-14 | STR | 19.30292 | 166.59483 | 18.6 | 1 | 0 |
| WAK_OCEAN_021 | 16-Mar-14 | STR | 19.31616 | 166.59858 | 5.5 | 0 | 1 |
| WAK-21 | 17-Mar-14 | ARMS | 19.31625 | 166.59824 | 13.7 | 0 | 3 |
| WAK-21 | 17-Mar-14 | BMU | 19.31625 | 166.59824 | 14.6 | 0 | 5 |
| WAK-21 | 17-Mar-14 | CAU | 19.31625 | 166.59824 | 14.6 | 0 | 5 |
| WAK-21 | 17-Mar-14 | CAU | 19.31625 | 166.59824 | 13.4 | 4 | 0 |
| WAK-22 | 17-Mar-14 | CAU | 19.29182 | 166.60728 | 12.8 | 0 | 5 |
| WAK-22 | 17-Mar-14 | CAU | 19.29182 | 166.60728 | 3.4 | 5 | 0 |
| WAK_OCEAN_004 | 17-Mar-14 | STR | 19.30616 | 166.65108 | 14.3 | 0 | 1 |
| WAK_OCEAN_004 | 17-Mar-14 | STR | 19.30616 | 166.65108 | 14.6 | 1 | 0 |
| WAK_OCEAN_005 | 17-Mar-14 | STR | 19.30623 | 166.65148 | 25.3 | 1 | 1 |
| WAK_OCEAN_017 | 17-Mar-14 | STR | 19.31620 | 166.62731 | 20.7 | 1 | 0 |
| WAK_OCEAN_023 | 17-Mar-14 | STR | 19.31602 | 166.62721 | 14 | 0 | 1 |
| WAK_OCEAN_024 | 17-Mar-14 | STR | 19.30637 | 166.65061 | 5.5 | 0 | 1 |
| WAK_OCEAN_031 | 17-Mar-14 | STR | 19.31576 | 166.62687 | 5.5 | 0 | 1 |
| WAK_OCEAN_032 | 17-Mar-14 | STR | 19.31620 | 166.62721 | 25 | 0 | 1 |
| WAK_OCEAN_009 | 18-Mar-14 | SST-STR | 19.30735 | 166.62209 | 0.3 | 1 | 0 |
| WAK_OCEAN_001 | 18-Mar-14 | STR | 19.28033 | 166.62866 | 12.2 | 1 | 0 |
| WAK_OCEAN_003 | 18-Mar-14 | STR | 19.30863 | 166.60449 | 1.5 | 1 | 1 |
| WAK_OCEAN_008 | 18-Mar-14 | STR | 19.28868 | 166.61361 | 1.2 | 0 | 1 |
| WAK_OCEAN_009 | 18-Mar-14 | STR | 19.30735 | 166.62209 | 2.7 | 1 | 0 |
| WAK_OCEAN_013 | 18-Mar-14 | STR | 19.27674 | 166.65721 | 20.7 | 1 | 0 |
| WAK-09 | 19-Mar-14 | ARMS | 19.27069 | 166.65155 | 13.7 | 3 | 0 |
| WAK-23 | 19-Mar-14 | BMU | 19.31602 | 166.62721 | 13.7 | 0 | 5 |
| WAK-24 | 19-Mar-14 | BMU | 19.30616 | 166.65108 | 14.3 | 0 | 5 |
| WAK-09 | 19-Mar-14 | CAU | 19.27069 | 166.65155 | 13.7 | 5 | 0 |
| WAK-23 | 19-Mar-14 | CAU | 19.31602 | 166.62721 | 13.7 | 0 | 5 |
| WAK-24 | 19-Mar-14 | CAU | 19.30616 | 166.65108 | 14.3 | 0 | 5 |
| WAK-13 | 20-Mar-14 | CAU | 19.31539 | 166.64336 | 13.7 | 5 | 0 |

## B.2. Benthic Environment

REA benthic sampling utilized a two-stage stratified random sampling design that incorporated three depth categories [shallow (0–6 m), mid (> 6–18 m), and deep (> 18–30 m)] to define strata. Belt-transect surveys and photo quadrats were conducted at 20 REA sites around Wake Atoll to assess coral populations, benthic community structure and composition, and coral and algal disease; water samples for microbial analyses were collected at 4 REA sites (Fig. B.2.1 and Table B.2.1). For more information about collections made at REA sites, see Table I.1.1 in Appendix I: "Biological Collections."

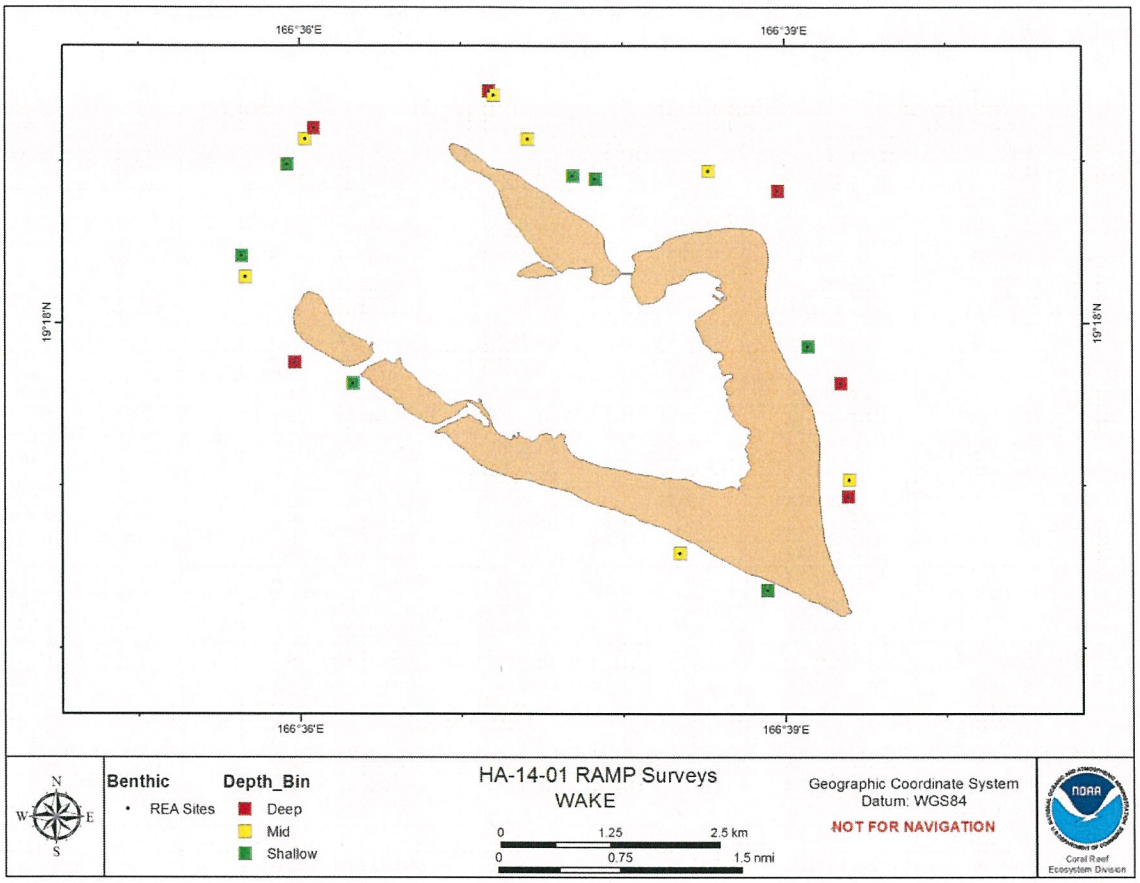


**Figure B.2.1.--**Locations of REA benthic sites surveyed at Wake Atoll during cruise HA-14-01. All of these REA sites were selected using a stratified random design

**Table B.2.1.**--Summary of the REA benthic surveys and microbial water collections performed at Wake Atoll during cruise HA-14-01.

| REA Site | Date | Depth Bin | Reef Zone | Depth (ft) | Latitude | Longitude | REA Coral Survey | Microbial Samples |
|---|---|---|---|---|---|---|---|---|
| WAK-430 | 16-Mar-14 | Mid | Forereef | 31 | 19.27633 | 166.63918 | x | |
| WAK-417 | 17-Mar-14 | Deep | Forereef | 71 | 19.29594 | 166.59952 | x | |
| WAK-412 | 17-Mar-14 | Mid | Forereef | 47 | 19.30471 | 166.59445 | x | 7 |
| WAK-408 | 17-Mar-14 | Mid | Forereef | 51 | 19.31889 | 166.60060 | x | |
| WAK-426 | 17-Mar-14 | Shallow | Forereef | 23 | 19.31631 | 166.59878 | x | |
| WAK-1999 | 17-Mar-14 | NA | NA | NA | 19.29192 | 166.59406 | – | 1 |
| WAK-1998 | 18-Mar-14 | NA | NA | NA | 19.32430 | 166.59659 | – | 1 |
| WAK-436 | 18-Mar-14 | Deep | Forereef | 80 | 19.32385 | 166.61960 | x | |
| WAK-369 | 18-Mar-14 | Mid | Forereef | 48 | 19.31886 | 166.62356 | x | 8 |
| WAK-360 | 18-Mar-14 | Mid | Forereef | 53 | 19.32338 | 166.62006 | x | |
| WAK-404 | 18-Mar-14 | Mid | Forereef | 40 | 19.31558 | 166.64222 | x | |
| WAK-419 | 18-Mar-14 | Deep | Forereef | 60 | 19.31355 | 166.64928 | x | |
| WAK-392 | 18-Mar-14 | Shallow | Forereef | 20 | 19.31481 | 166.63050 | x | |
| WAK-388 | 18-Mar-14 | Shallow | Forereef | 18 | 19.31511 | 166.62820 | x | |
| WAK-420 | 16-Mar-14 | Mid | Forereef | 45 | 19.27310 | 166.64460 | – | 3 |
| WAK-413 | 19-Mar-14 | Deep | Forereef | 84 | 19.28219 | 166.65655 | x | |
| WAK-429 | 19-Mar-14 | Mid | Forereef | 51 | 19.28386 | 166.65669 | x | |
| WAK-363 | 19-Mar-14 | Shallow | Forereef | 18 | 19.27252 | 166.64818 | x | |
| WAK-383 | 19-Mar-14 | Shallow | Forereef | 16 | 19.29380 | 166.60555 | x | |
| WAK-395 | 19-Mar-14 | Shallow | Forereef | 16 | 19.30687 | 166.59408 | x | |
| WAK-434 | 19-Mar-14 | Shallow | Forereef | 24 | 19.29381 | 166.65584 | x | |
| WAK-384 | 19-Mar-14 | Shallow | Forereef | 71 | 19.29758 | 166.65244 | x | |
| WAK-433 | 20-Mar-14 | Deep | Forereef | 71 | 19.32003 | 166.60151 | x | |
| WAK-13 | 20-Mar-14 | Mid | Forereef | 45 | 19.31539 | 166.64336 | – | 7 |

CRED also completed 10 towed-diver surveys at Wake Atoll, covering a total length of 21 km (an area of 21 ha) on the ocean floor (Fig. B.2.2). The mean survey length was 2.0 km with a range of 1.4–2.5 km. The mean survey depth was 14.5 m with a range of 12.5–17.2 m. The mean temperature from data recorded during these surveys was 26.1°C with a range of 25.4°C–26.6°C.

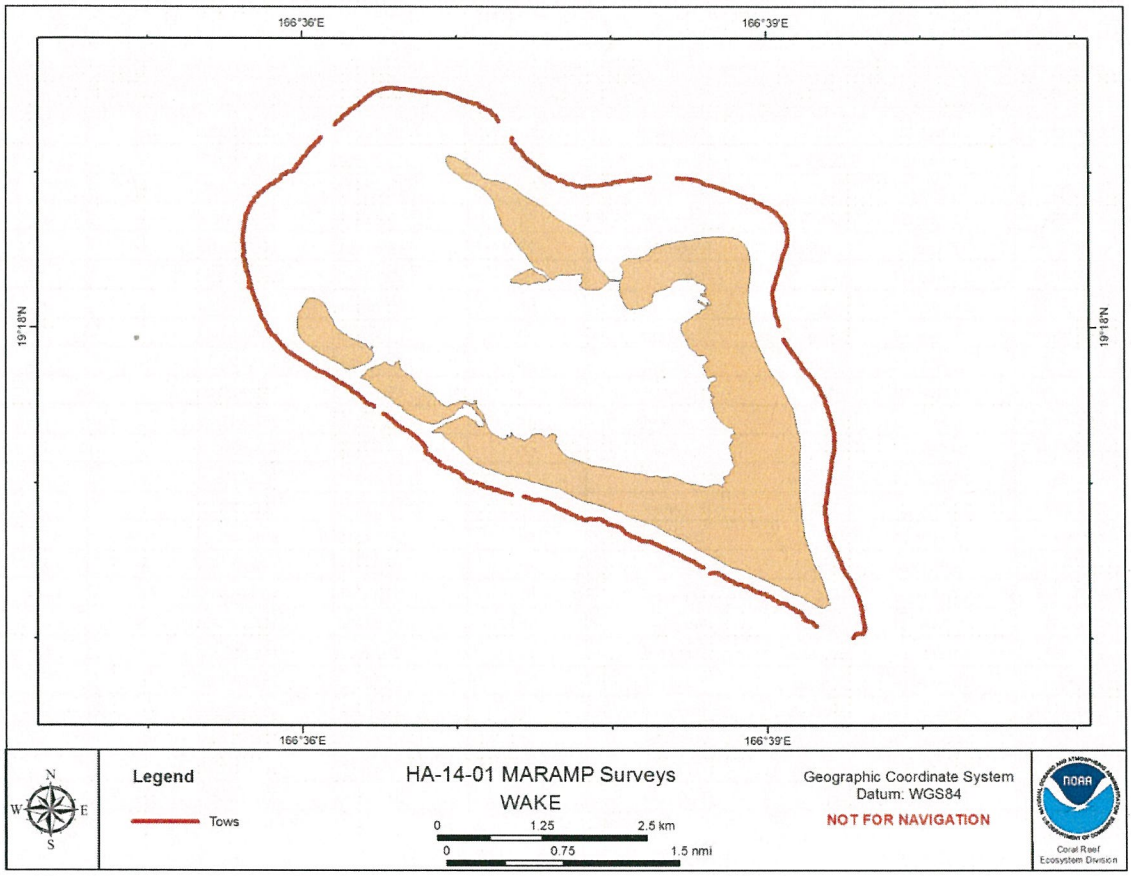


**Figure B.2.2.--**Track locations of towed-diver surveys conducted at Wake Atoll during the cruise HA-14-01.

## B.3. Reef Fish Community

REA fish survey sites were chosen using a stratified random design. Stationary-point-count surveys were conducted at 45 REA sites at Hawai`i Island over 3 different habitat strata: deep, moderate, and shallow forereef (Fig.B.3.1 and Table B.3.1). No fishes were collected during these surveys.

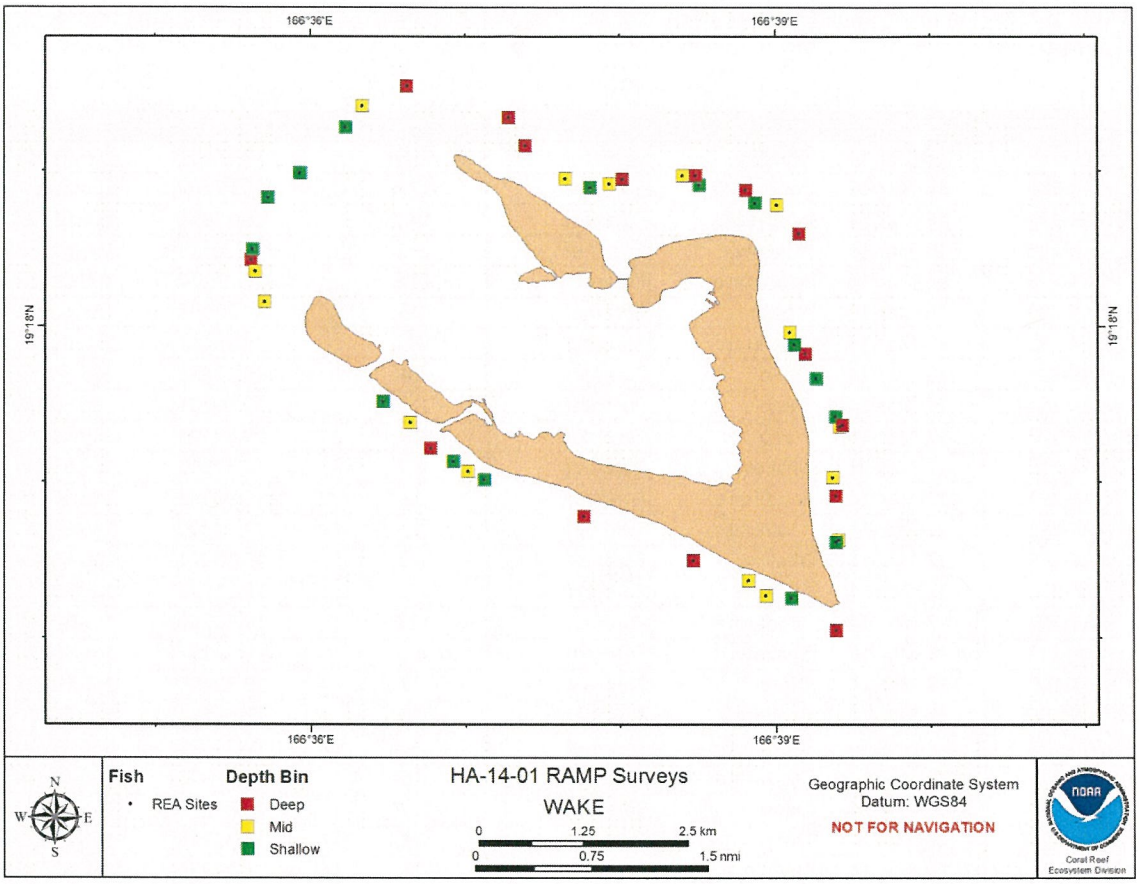


**Figure B.3.1.--**Locations of REA fish sites surveyed at Wake Atoll during cruise HA-14-01. All of these REA sites were selected using a stratified random sampling design.

**Table B.3.1.--**Summary of sites where REA fish surveys were conducted at Wake Atoll during cruise HA-14-01.

| REA_Site | Date | Depth Bin | Reef Zone | Depth (m) | Latitude | Longitude |
|---|---|---|---|---|---|---|
| WAK-153 | 16-Mar-14 | Shallow | Forereef | 3.5 | 19.28551 | 166.61541 |
| WAK-168 | 16-Mar-14 | Moderate | Forereef | 17.6 | 19.27107 | 166.64897 |
| WAK-174 | 16-Mar-14 | Moderate | Forereef | 14.2 | 19.28961 | 166.61080 |
| WAK-236 | 16-Mar-14 | Shallow | Forereef | 5.1 | 19.28353 | 166.61868 |
| WAK-246 | 16-Mar-14 | Moderate | Forereef | 13.8 | 19.27270 | 166.64712 |
| WAK-280 | 16-Mar-14 | Deep | Forereef | 23 | 19.27958 | 166.62946 |
| WAK-285 | 16-Mar-14 | Moderate | Forereef | 13.4 | 19.28441 | 166.61696 |
| WAK-301 | 16-Mar-14 | Deep | Forereef | 24 | 19.27485 | 166.64117 |
| WAK-154 | 17-Mar-14 | Deep | Forereef | 21.8 | 19.30706 | 166.59371 |
| WAK-172 | 17-Mar-14 | Deep | Forereef | 23 | 19.32573 | 166.61042 |
| WAK-176 | 17-Mar-14 | Shallow | Forereef | 4.7 | 19.31640 | 166.59894 |
| WAK-192 | 17-Mar-14 | Moderate | Forereef | 13 | 19.30582 | 166.59415 |
| WAK-252 | 17-Mar-14 | Moderate | Forereef | 13 | 19.32360 | 166.60567 |
| WAK-263 | 17-Mar-14 | Shallow | Forereef | 2.7 | 19.30821 | 166.59384 |
| WAK-266 | 17-Mar-14 | Shallow | Forereef | 5 | 19.31375 | 166.59548 |
| WAK-267 | 17-Mar-14 | Moderate | Forereef | 13.6 | 19.30260 | 166.59513 |
| WAK-302 | 17-Mar-14 | Shallow | Forereef | 4.6 | 19.32132 | 166.60385 |
| WAK-166 | 18-Mar-14 | Deep | Forereef | 26 | 19.31456 | 166.64688 |
| WAK-180 | 18-Mar-14 | Moderate | Forereef | 16 | 19.31614 | 166.64010 |
| WAK-183 | 18-Mar-14 | Moderate | Forereef | 13.4 | 19.31525 | 166.63226 |
| WAK-189 | 18-Mar-14 | Moderate | Forereef | 16 | 19.31300 | 166.65018 |
| WAK-199 | 18-Mar-14 | Deep | Forereef | 22.7 | 19.31573 | 166.63363 |
| WAK-205 | 18-Mar-14 | Deep | Forereef | 22 | 19.31936 | 166.62320 |
| WAK-241 | 18-Mar-14 | Deep | Forereef | 21 | 19.32235 | 166.62142 |
| WAK-258 | 18-Mar-14 | Moderate | Forereef | 14 | 19.31579 | 166.62756 |
| WAK-265 | 18-Mar-14 | Deep | Forereef | 24.2 | 19.31611 | 166.64151 |
| WAK-272 | 18-Mar-14 | Shallow | Forereef | 5.5 | 19.31482 | 166.63015 |
| WAK-278 | 18-Mar-14 | Shallow | Forereef | 6 | 19.31318 | 166.64788 |
| WAK-287 | 18-Mar-14 | Shallow | Forereef | 6 | 19.31512 | 166.64190 |
| WAK-147 | 19-Mar-14 | Moderate | Forereef | 13 | 19.27703 | 166.65680 |
| WAK-160 | 19-Mar-14 | Deep | Forereef | 26 | 19.30990 | 166.65259 |
| WAK-171 | 19-Mar-14 | Moderate | Forereef | 14.2 | 19.28921 | 166.65695 |
| WAK-198 | 19-Mar-14 | Moderate | Forereef | 14.8 | 19.29931 | 166.65158 |
| WAK-204 | 19-Mar-14 | Moderate | Forereef | 11 | 19.28371 | 166.65623 |
| WAK-206 | 19-Mar-14 | Shallow | Forereef | 5.5 | 19.29025 | 166.65652 |
| WAK-208 | 19-Mar-14 | Deep | Forereef | 27.9 | 19.28936 | 166.65727 |
| WAK-212 | 19-Mar-14 | Shallow | Forereef | 6.4 | 19.29794 | 166.65207 |
| WAK-226 | 19-Mar-14 | Deep | Forereef | 20 | 19.26738 | 166.65655 |
| WAK-242 | 19-Mar-14 | Shallow | Forereef | 6 | 19.29435 | 166.65449 |
| WAK-259 | 19-Mar-14 | Deep | Forereef | 22.6 | 19.29699 | 166.65328 |
| WAK-275 | 19-Mar-14 | Shallow | Forereef | 3.3 | 19.27082 | 166.65170 |
| WAK-281 | 19-Mar-14 | Shallow | Forereef | 5.5 | 19.27682 | 166.65657 |
| WAK-289 | 19-Mar-14 | Deep | Forereef | 23.9 | 19.28175 | 166.65653 |
| WAK-167 | 20-Mar-14 | Shallow | Forereef | 5 | 19.29191 | 166.60786 |
| WAK-182 | 20-Mar-14 | Deep | Forereef | 24 | 19.28686 | 166.61295 |

# APPENDIX C: BIOLOGICAL COLLECTIONS

Biological and other samples were collected at Wake Atoll and surrounding waters for multiple research purposes. These collections are listed here in Table C.1.1.

**Table I.1.1.**--Samples collected at Wake Atoll for microbial and ocean acidification analyses during cruise HA-14-01.

| REA Site | Date | Latitude | Longitude | Specimen Collected | Number of Samples | Depth (m) |
|---|---|---|---|---|---|---|
| Microbial Collections: Water Samples, Coral Rubble, and Macroalgae | | | | | | |
| WAK-420 | 16-Mar-14 | 19.27310 | 166.64460 | 2 L | 3 | 13.7 |
| WAK-1999 | 17-Mar-14 | 19.29192 | 166.59406 | 2 L | 1 | |
| WAK-412 | 17-Mar-14 | 19.30471 | 166.59445 | 2 L | 3 | 13.7 |
| WAK-412 | 17-Mar-14 | 19.30471 | 166.59445 | 20 L | 4 | 13.7 |
| WAK-1998 | 18-Mar-14 | 19.32430 | 166.59659 | 2 L | 1 | |
| WAK-369 | 18-Mar-14 | 19.31886 | 166.62356 | 2 L | 3 | 13.7 |
| WAK-369 | 18-Mar-14 | 19.31886 | 166.62356 | Coral rubble | 5 | 13.7 |
| WAK-13 | 20-Mar-14 | 19.31539 | 166.64336 | 2 L | 3 | 12.2 |
| WAK-13 | 20-Mar-14 | 19.31539 | 166.64336 | 20 L | 4 | 12.2 |
| Algal Collections: Ocean Acidification | | | | | | |
| WAK-21 | 17-Mar-14 | 19.31625 | 166.59824 | CAU unit | 4 | 13.4 |
| WAK-20 | 16-Mar-14 | 19.28065 | 166.62778 | CAU unit | 5 | 14.6 |
| WAK-22 | 17-Mar-14 | 19.29182 | 166.60728 | CAU unit | 5 | 3.4 |
| WAK-09 | 19-Mar-14 | 19.27069 | 166.65155 | CAU unit | 5 | 13.7 |
| WAK-13 | 20-Mar-14 | 19.31539 | 166.64336 | CAU unit | 5 | 13.7 |