## Appendix I. Methods: Ecological Baselines for Climate Change

At each Climate Monitoring site, one subsurface temperature recorder (STR), five Calcification Accretion Units (CAUs) and three Autonomous Reef Monitoring Structures (ARMS) were typically deployed (Figure 113). In 2012, 10 Climate Monitoring sites were established; in 2014, eight Climate Monitoring sites were revisited and instruments were recovered.

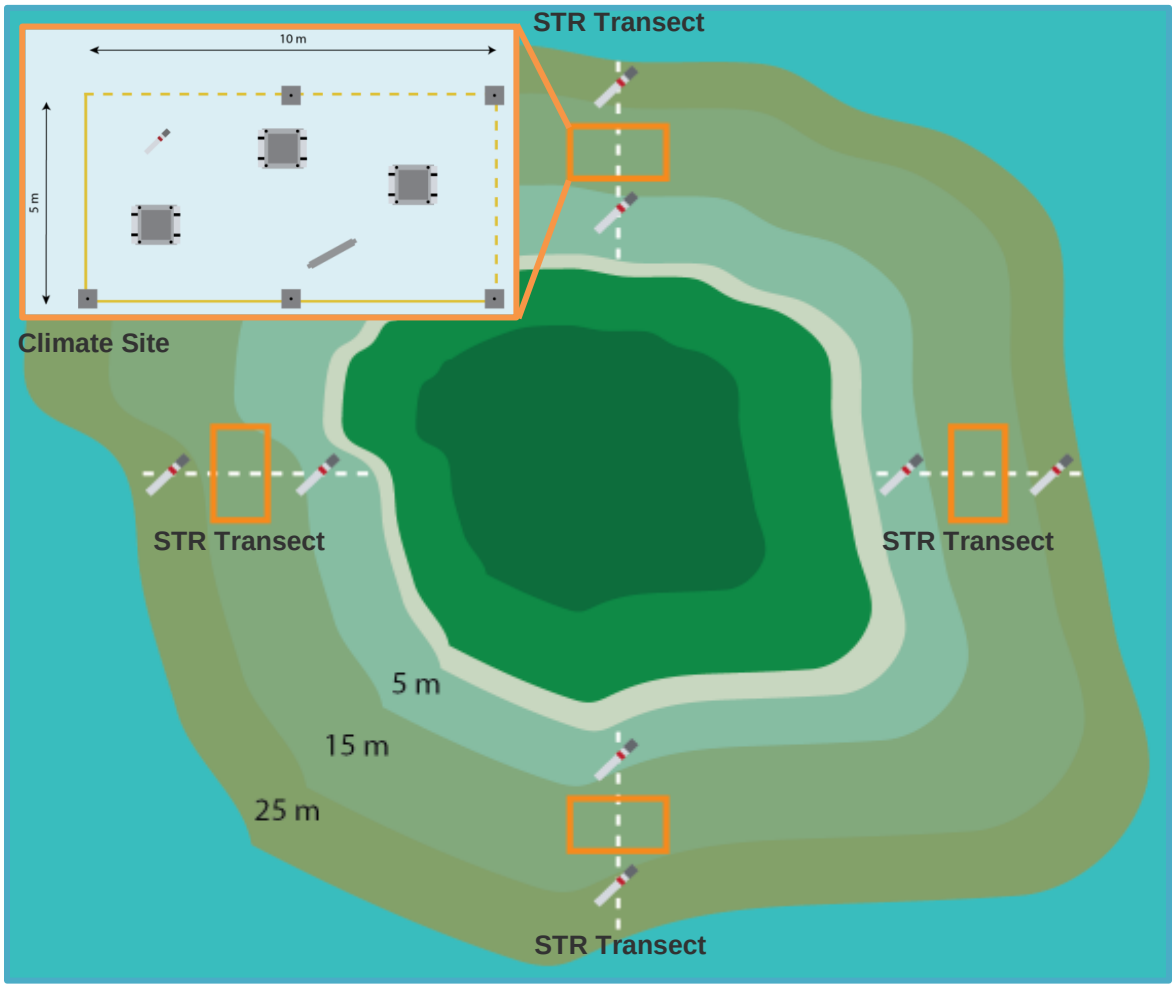


**Figure 113.** Schematic of Climate Monitoring sites (orange boxes) and STR transects (dashed white lines) around an island. A STR transect includes 1 STR deployed in the shallow (0–6 m), mid (6–18 m), and deep (18–30 m) water at each site, and the mid-depth STR is deployed within the Climate Monitoring site. Inset shows details of the Climate Monitoring site with the deployment of three ARMS (large grey squares), five CAUs (small grey squares), one STR (top left), one water sample (bottom middle), and the photoquad (dashed orange line).

### *Temperature (STRs)*

#### *Field Method*

Temperature data were collected using high-accuracy, subsurface temperature recorders (STRs) made by Sea-Bird Electronics (model no. 39), which sampled at a rate of 1 temperature measurement every 60 minutes throughout the 2-year deployment. STRs were attached to a mounting bracket with weights and then strapped to reef substrate at the benthos using large cable ties. At each of the Climate Monitoring sites, one STR was deployed in the mid-depth range (6–18 m)in close proximity to the other instruments. Additionally, STR transects (Figure 113) were established at 4 of the sites in 2012, with a second STR deployed in the shallow depth range (0–6 m), and a third STR deployed in the deep water range (18–30 m) at 3 of those 4 sites. See Table 23 for a complete list of STRs deployed and recovered.

#### *Remote Sensing Method*

To serve as a comparison to the STR data, we extracted the NOAA ¼° daily Optimum Interpolation Sea Surface Temperature (OISST) for the northern Timor-Leste region. The OISST is an analysis constructed by combining temperature observations from different platforms (satellites, ships, and buoys) on a regular global grid (Reynolds et al. 2002).

#### *Analysis*

For each STR, there are two versions of the data files with .ASC and .CDP extensions. Data downloaded from the instruments were saved as an ASC file and include the header information. The data were then processed and quality controlled using MATLAB to remove the header, extraneous data from the periods prior to deployment and post recovery, and erroneous records, and were then saved as a CDP (CREP Data Product) text file.

Raw STR data were smoothed with a 180-day running mean reducing the associated daily variability for visualization purposes and highlighting the main temperature patterns. Daily OISST data were smoothed using a 7-day running mean for the same purpose.

**Table 23.** STRs deployed and recovered between 2012 and 2014 by NOAA-CREP in Timor-Leste. No data were collected from the mid-depth STR recovered at LAU-01 (record in grey).

| SITE ID | LATITUDE | LONGITUDE | DEPTH (m) | DEPLOY DATE | RECOVER DATE |
|---|---|---|---|---|---|
| BOB-02 | -8.85328816 | 125.0132672 | 14.9 | 10/17/2012 | 9/22/2014 |
| DIL-02 | -8.55484044 | 125.4992917 | 6.1 | 10/15/2012 | 10/9/2014 |
| DIL-02 | -8.55465126 | 125.4991272 | 14.0 | 10/15/2012 | 10/9/2014 |
| DIL-02 | -8.55459971 | 125.4989724 | 24.7 | 10/15/2012 | – |
| VIL-03 | -8.30331881 | 125.5584685 | 13.1 | 10/18/2012 | 9/16/2014 |
| VIL-10 | -8.22428752 | 125.6167984 | 6.1 | 10/25/2012 | 9/18/2014 |
| VIL-10 | -8.22440553 | 125.6168395 | 13.1 | 10/25/2012 | 9/18/2014 |
| VIL-10 | -8.22448508 | 125.6168435 | 25.0 | 10/25/2012 | 9/18/2014 |
| MAF-01 | -9.15203 | 125.8220562 | 14.6 | 10/21/2012 | – |
| MAN-09 | -8.47532158 | 125.9065769 | 4.9 | 10/24/2012 | 9/24/2014 |
| MAN-09 | -8.47513382 | 125.9067561 | 14.6 | 10/24/2012 | 9/24/2014 |
| BAU-04 | -8.41960078 | 126.4270697 | 12.8 | 10/19/2012 | 9/28/2014 |
| LAU-07 | -8.6842867 | 126.9897779 | 11.0 | 10/22/2012 | – |
| LAU-01 | -8.34661318 | 127.1611709 | 4.6 | 10/23/2012 | 10/6/2014 |
| LAU-01 | -8.3463836 | 127.160992 | 14.3 | 10/23/2012 | 10/6/2014 |
| LAU-01 | -8.34633071 | 127.1609347 | 25.3 | 10/23/2012 | 10/6/2014 |
| LAU-05 | -8.41080515 | 127.3122215 | 14.6 | 10/20/2012 | 10/3/2014 |

### *Seawater Chemistry*

#### *Field Method*

At each Climate Monitoring site, 1 discrete near reef seawater sample (recovered at ~15-m depth) and 1 surface seawater sample (recovered at ~1-m depth) were collected using 5-L Niskin bottles. In 2013, a third seawater sample was collected ~1-km offshore from each site (recovered at ~1-m depth). See Table 24 for a complete list of water samples collected in 2013 and 2014. Each time a water sample was collected, it was divided into: (1) a 500-mL glass bottle and preserved with mercuric chloride (for dissolved inorganic carbon [DIC] and total alkalinity [TA] analysis) and (2) a 250-mL HDPE plastic bottle (for salinity analysis). During both 2013 and 2014 field efforts, 1 in 4 water sample collections were replicated to ensure analytical reproducibility.

**Table 24.** Water samples collected around Timor-Leste in 2013 and 2014. Three water samples (surface, benthic, and offshore) were collected at eight of the Climate Monitoring sites in 2013 and additional samples were collected at a subset of the reef fish survey sites. Two water samples (surface and benthic only) were collected at the same eight Climate Monitoring sites in 2014.

| SITE ID | LATITUDE | LONGITUDE | DATE | SURFACE (m) | BENTHIC (m) | OFFSHORE (m) |
|---|---|---|---|---|---|---|
| **2013 Climate Monitoring Sites** | | | | | | |
| BOB-02 | -8.85324089 | 125.0132288 | 6/14/2013 | 0.9 | 14.0 | |
| BOB-02 | -8.8474899 | 125.0066692 | 6/14/2013 | | | 0.9 |
| DIL-02 | -8.55469116 | 125.4992045 | 6/27/2013 | 0.9 | 14.0 | |
| DIL-02 | -8.54703639 | 125.4959428 | 6/27/2013 | | | 0.9 |
| VIL-03 | -8.3033375 | 125.5585172 | 6/6/2013 | 0.9 | 15.4 | |
| VIL-03 | -8.3089315 | 125.5526682 | 6/6/2013 | | | 0.9 |
| VIL-10 | -8.22437125 | 125.6168803 | 6/6/2013 | 0.9 | 13.7 | |
| VIL-10 | -8.22253654 | 125.623466 | 6/6/2013 | | | 0.9 |
| MAN-09 | -8.47521907 | 125.9067249 | 6/19/2013 | 0.9 | 11.6 | |
| MAN-09 | -8.46729632 | 125.9064028 | 6/19/2013 | | | 0.9 |
| BAU-04 | -8.41959751 | 126.4270859 | 6/20/2013 | 0.9 | 14.3 | |
| BAU-04 | -8.41341812 | 126.4302182 | 6/20/2013 | | | 0.9 |
| LAU-01 | -8.34639223 | 127.1610254 | 6/21/2013 | 0.9 | 14.3 | |
| LAU-01 | -8.33927021 | 127.1554364 | 6/21/2013 | | | 0.9 |
| LAU-05 | -8.4108282 | 127.3121913 | 6/25/2013 | 0.9 | 16.8 | |
| LAU-05 | -8.40311006 | 127.311871 | 6/25/2013 | | | 0.9 |
| **2013 Other (non-climate) Sites** | | | | | | |
| OEC-43 | -9.19571104 | 124.372808 | 6/16/2013 | 0.9 | 13.1 | |
| OEC-43 | -9.188659 | 124.3683361 | 6/16/2013 | | | 0.9 |
| ATA-62 | -8.15301334 | 125.6024217 | 6/18/2013 | | 13.7 | |
| DIL-12 | -8.51264996 | 125.74406 | 6/27/2013 | | 12.5 | |
| LAU-29 | -8.36221207 | 127.075241 | 6/26/2013 | | 11.3 | |
| LAU-03 | -8.36288187 | 127.1034242 | 6/26/2013 | | 11.3 | |
| LAU-09 | -8.38678312 | 127.2780601 | 6/25/2013 | | 12.8 | |
| **2014 Climate Monitoring Sites** | | | | | | |
| BOB-02 | -8.85320778 | 125.0131945 | 9/22/2014 | 0.9 | 14.6 | |
| DIL-02 | -8.55465 | 125.49912 | 10/9/2014 | 0.9 | 13.7 | |
| VIL-03 | -8.303372115 | 125.5584964 | 9/16/2014 | 0.9 | 14.9 | |
| VIL-10 | -8.224377288 | 125.6168642 | 9/18/2014 | 0.9 | 13.1 | |
| MAN-09 | -8.475181097 | 125.9066781 | 9/24/2014 | 0.9 | 14.6 | |
| BAU-04 | -8.41943 | 126.4268 | 9/28/2014 | 0.9 | 14.3 | |
| LAU-01 | -8.34643 | 127.16095 | 10/6/2014 | 0.9 | 14.6 | |
| LAU-05 | -8.41082 | 127.31222 | 10/3/2014 | 0.9 | 15.5 | |

In 2013, electronic measurements of temperature and pressure were taken at the location where each water sample was collected using a Seabird SBE-39 subsurface temperature recorder. In 2014, immediately upon returning to the dive boat, a conductivity-temperature-depth instrument was used to sample through the water column above the 15-m survey site using a SBE-19plus.

*Analysis*

Water samples were shipped to the NOAA Pacific Marine Environmental Laboratory (PMEL) in Seattle, WA for laboratory analysis of dissolved inorganic carbon, total alkalinity, and salinity.

See PMEL's methodology to collect seawater samples for carbonate chemistry analysis (http://www.pmel.noaa.gov/co2/files/dic_sample_technique_revised_5-17-10.pdf).

### *Calcification Accretion Units (CAUs)*

*Field Method*

The following description was adapted from the methods section of Vargas-Angel et al. (2015). Each CAU assembly comprised two 10-cm × 10-cm PVC plates separated by a 1-cm plastic spacer and mounted on a stainless steel all-thread rod. These assemblies were attached to a stainless steel stake installed into hard substrate around the perimeter of each climate survey site, and left to accrete for approximately 2 years (Figure 114). During recovery, each CAU was placed in a Ziploc bag to minimize the loss of attached calcified material during transport. Recovered CAUs were frozen at –5°C for preservation during transportation to the laboratory in Honolulu, Hawaii. See Table 25 for a complete list of CAUs deployed and recovered.

**Figure 114.** Newly deployed Calcification Accretion Unit (CAU) on the seafloor at one of the Climate Monitoring sites in Timor-Leste (*left*). CAUs installed within a Climate Monitoring site with an STR deployed nearby (*middle).* CAU about to be recovered approximately two years later (*right*).

**Table 25.** CAUs deployed and recovered between 2012 and 2014 in Timor-Leste.

| SITE ID | LATITUDE | LONGITUDE | DEPTH (m) | DEPLOY DATE | NUMBER DEPLOYED | RECOVER DATE | NUMBER RECOVERED | SOAK TIME (days) |
|---|---|---|---|---|---|---|---|---|
| BOB-02 | -8.85329 | 125.01327 | 14.0 | 10/17/2012 | 5 | 9/22/2014 | 4 | 705 |
| DIL-02 | -8.55465 | 125.49913 | 13.1 | 10/15/2012 | 5 | 10/9/2014 | 5 | 724 |
| VIL-03 | -8.30332 | 125.55847 | 14.3 | 10/18/2012 | 5 | 9/16/2014 | 5 | 698 |
| VIL-10 | -8.22441 | 125.61684 | 13.1 | 10/25/2012 | 5 | 9/18/2014 | 4 | 693 |
| MAF-01 | -9.15203 | 125.82206 | 14.6 | 10/21/2012 | 5 | – | – | – |
| MAN-09 | -8.47513 | 125.90675 | 14.6 | 10/24/2012 | 5 | 9/24/2014 | 1 | 700 |
| BAU-04 | -8.4196 | 126.42707 | 13.7 | 10/19/2012 | 5 | 9/28/2014 | 5 | 709 |
| LAU-07 | -8.68429 | 126.98978 | 11.0 | 10/22/2012 | 5 | – | – | – |
| LAU-01 | -8.34638 | 127.161 | 14.6 | 10/23/2012 | 5 | 10/6/2014 | 5 | 713 |
| LAU-05 | -8.4108 | 127.31222 | 14.6 | 10/20/2012 | 5 | 10/3/2014 | 5 | 713 |

*Analysis*

In the laboratory, after disassembly of each CAU, plates were dried at 60°C for 2–5 days, and were classified as dry when the difference in weight between sequential weighings was less than 0.1 g. After drying, each individual plate was submerged in a 5% hydrochloric acid bath (HCl) for 24-hours or until all calcium carbonate ($CaCO_3$) had dissolved. As the HCl solution was neutralized by the $CaCO_3$ dissolution (indicated by the absence of gas bubbles), additional HCl was added to complete the dissolution process. Often, the addition of acid was repeated several times in a 24–72-hour period until all $CaCO_3$ was removed. The remaining fleshy tissue was scraped onto pre-weighed 11-µm cellulose filter paper, vacuum filtered along with all 5% HCl supernatant from the dissolution process, and dried at 60°C until constant weight using the same dryness criteria above; 48 hours minimum. The clean, scraped, and dried CAU plates were re-weighed, and the mass of $CaCO_3$ was determined by subtracting the combined weight of the fleshy tissue and PVC plates from the initial dry weight of the CAU prior to dissolution. To determine the rate of $CaCO_3$ accretion, the mass of $CaCO_3$ was normalized for surface area of each CAU (400 $cm^2$—accounting for all upper and lower plate surfaces) and the amount of time in days that each CAU was deployed, rendering a measure of net $CaCO_3$ accretion in units of g $CaCO_3$ $cm^{-2}$ $yr^{-1}$. This reef calcification rate was averaged between the CAU units recovered at each site.

### *Autonomous Reef Monitoring Structure (ARMS)*

*Field Method*

ARMS, composed of nine PVC plates (23 cm x 23 cm) stacked in alternating series of open and semi-enclosed layers, were affixed to the seafloor between 12–15 m in replicate sets of three (Figure 115A). They remained on the benthos for two years during which time they were naturally colonized with marine organisms (Figure 115B). After the 2-year deployment period, the ARMS units were encapsulated within a 106-µm nitex-lined crate, brought to the surface, placed within a large seawater holding bin and transported to shore. On shore, they were disassembled plate by plate, with both sides

photo-documented (see the ARMS plate collages in Appendix E). The plates were then scraped clear of all the accumulated sessile biomass and immediately homogenized in a blender, filtered with a 40-µm net, subsampled, and preserved for metabarcoding (Figure 115C).

The seawater used during processing was sieved using 2-mm, 500-µm and 106-µm geologic sieves to create three size fractions (Figure 115D). The >2 mm fraction was sorted to morphospecies, photographed, and brachyuran crabs were preserved for DNA barcoding (Figure 115E). The two smaller motile fractions were preserved for additional lab and molecular processing. See Table 26 for a complete list of ARMS deployed and recovered.


**Figure 115.** Clockwise starting from upper left: A) SCUBA diver attaching an ARMS to the seafloor; B) An ARMS about to be recovered after deployment for ~2 years; C) Scraping an ARMS plate; D) A sieved 2-mm fraction; E) Sorting through the organisms recovered from the 2-mm fraction.

**Table 26.** ARMS deployed and recovered in Timor-Leste from 2012 to 2014.

| SITE ID | LATITUDE | LONGITUDE | DEPTH (m) | DEPLOY DATE | NUMBER DEPLOYED | RECOVER DATE | NUMBER RECOVERED |
|---|---|---|---|---|---|---|---|
| BOB-02 | -8.85329 | 125.01327 | 14.0 | 10/17/2012 | 3 | 9/22/2014 | 3 |
| DIL-02 | -8.55465 | 125.49913 | 13.1 | 10/15/2012 | 4 | 10/9/2014 | 3 |
| VIL-03 | -8.30332 | 125.55847 | 14.3 | 10/18/2012 | 3 | 9/16/2014 | 3 |
| VIL-10 | -8.22441 | 125.61684 | 13.1 | 10/25/2012 | 3 | 9/17/2014 | 3 |
| MAF-01 | -9.15203 | 125.82206 | 14.6 | 10/21/2012 | 3 | – | – |
| MAN-09 | -8.47513 | 125.90675 | 14.6 | 10/24/2012 | 3 | 9/24/2014 | 3 |
| BAU-04 | -8.4196 | 126.42707 | 13.7 | 10/19/2012 | 3 | 9/28/2014 | 3 |
| LAU-07 | -8.68429 | 126.98978 | 11.0 | 10/22/2012 | 3 | – | – |
| LAU-01 | -8.34638 | 127.161 | 14.6 | 10/23/2012 | 3 | 10/6/2014 | 3 |
| LAU-05 | -8.4108 | 127.31222 | 14.6 | 10/20/2012 | 4 | 10/3/2014 | 4 |

*Lab Methods*

***Decantation***—Due to sediment within the 500-µm and 106-µm fractions that can inhibit metabarcoding laboratory processing, a decantation procedure was conducted on these fractions from each ARMS unit to separate the sediment from the organic matter (Leray and Knowlton 2015). Upon the completion of the decantation process, half of the sample was crushed with a mortar and pestle for DNA extraction and metabarcoding while the other half was preserved as a backup.

***DNA barcoding***—Legs from brachyuran crabs were subsampled, and genomic DNA was extracted using standard proteinase-k digestion followed by phenol-chloroform extraction on the AutoGenprep 965 (Autogen). Primers designed by Geller et al. (2013) were used to target approximately 658 base pairs of the COI gene and automated sequencing techniques were used to sequence in both directions.

***DNA metabarcoding***—DNA was extracted from 10 grams of the homogenized sessile scrapings and from the decanted 500-µm and 100-µm motile fractions using the MO-Bio PowerMax Soil extraction kits. Using the reverse primer, jgHCO2198 (Geller et al. 2013), and the forward primer, mlCOIintF (Leray et al. 2013), a 313 base pair fragment of COI was amplified using a PCR (polymerase chain reaction) touchdown protocol with 16 initial cycles: denaturation for 10 seconds at 95°C, annealing for 30 seconds at 62°C (–1°C per cycle), and extension for 60 seconds at 72°C, followed by 25 cycles at 46°C annealing temperature (Leray et al. 2013; Leray and Knowlton 2015). PCRs were performed in triplicates and inspected on agarose gels. Triplicate PCR products were pooled, cleaned using Agencourt AMPure beads, and quantified using Biotum AccuClear Ultra High Sensitivity Quantification Kit. PCR products were then inserted directly into the Kappa Systems Hyper-Prep sample kit using dual-end Illumina adapters for ligation. Sample libraries were validated by visualization on an Agilent 2100 BioAnalyzer, quantified using qPCR, pooled, and sequenced on an Illumina MiSeq platform. Each library yielded approximately 250,000 reads per sample, and a standard quality control filter was run to parse the Illumina reads into FASTQ files sorted by index.

*Analysis*

***Morphospecies (2-mm size fraction)***—Overall abundance of >2 mm organisms was averaged between ARMS units recovered at each site to give a site-level metric. Organisms were additionally averaged by ARMS unit at the island scale for comparison with other ARMS recovery locations across the Pacific. Dominant phyla and taxa groups within phyla were averaged between ARMS units and compared across sites.

***Crab DNA barcoding***—Resulting sequences of crabs were clustered into Operational Taxonomic Units (OTUs) and blasted (cross checked) against existing DNA-barcoding libraries (Barcode of Life Data Systems [BOLD] and Moorea Biocode). Matched sequences with >97% identity and >85% coverage were identified to an existing record of the species within the databases. Those crab sequences with <97% identity and >85% coverage underwent a phylogenetic Bayesian approach using the Statistical Assignment Package (SAP) to assign OTUs to higher taxonomic levels in the absence of a direct match. Species richness was averaged by ARMS unit at each site and examined on the island scale in relation to the richness of brachyuran crabs from other ARMS units collected by NOAA-CREP in the Pacific Ocean. Broad scale richness values were calculated per ARMS unit richness rather than by island due to the variability in the number of ARMS units deployed across islands.

***Metabarcoding bioinformatics***—Sequences were assembled, trimmed, cleaned, and dereplicated following standard bioinformatics techniques using available software programs. Dereplicated sequences were then aligned to COI barcodes from the BOLD database. Matched sequences ≥97% identity and ≥85% coverage are presented herein. Sequences that did not have a direct match have not been directly DNA barcoded and thus species resolution is not available. Once the phylogenetic approaches and bioinformatic software have been refined, the remaining unknown sequences can be determined. Currently available software is not capable of working through 10 million plus sequence reads that span across multiple phyla. However, through the efforts of a third-party bioinformation specialist working on these data sets for Timor-Leste, a solution will be found in the near future to provide phyla-based resolution of the remaining sequences that will indicate percent cover of the phyla communities that have recruited to the ARMS units.

### *Benthic Cover*

*Field Method*

At each Climate Monitoring site, digital photos of the benthos were collected at 1-m intervals along two transects implementing a high-resolution digital camera mounted on a pole. This process generated ~30 photographs per site.

*Analysis*

Benthic photographs were analyzed using CPCe following the same method as described in the *Analysis: Benthic Cover Derived from Analysis of Benthic Images Collected during Fish Surveys* section of Appendix G.