Second WESTPAC Training Workshop

“Research and Monitoring of the Ecological Impacts of Ocean Acidification on Coral Reef Ecosystems”

Phuket, Thailand, 26-28 August 2015

hosted by

In collaboration with
26-28 August 2015
Phuket, Thailand

Second WESTPAC Training Workshop on “Research and Monitoring of the Ecological Impacts of Ocean Acidification on Coral Reef Ecosystems”

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Provisional Programme
## Provisional Programme

### Tuesday 25 August 2015

Arrival of participants and check in at the Kantary Bay Hotel, Phuket

### Wednesday 26 August 2015

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<tr>
<th>Time</th>
<th>Activity</th>
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</thead>
<tbody>
<tr>
<td>08:30</td>
<td>Gathering at the lobby of the Kantary Bay Hotel, Phuket, and departure for the Phuket Marine Biological Center (PMBC)</td>
</tr>
<tr>
<td>08:45 – 09:00</td>
<td>Registration  &lt;br&gt; <strong>Venue:</strong> Meeting Room, on the second floor of the Phuket Aquarium, Phuket Marine Biological Center (PMBC)</td>
</tr>
<tr>
<td>09:00 – 09:20</td>
<td>Opening and self introduction  &lt;br&gt; - Welcome Remarks by Dr Somkiat Khokiattiwong, Acting Director of the Phuket Marine Biological Center (PMBC) and Chair of the IOC Sub-Commission for the Western Pacific (WESTPAC)  &lt;br&gt; - Welcome Remarks by Ms. Duriya Amatavivat, Deputy Secretary-General, The Thai National Commission for UNESCO  &lt;br&gt; - Participants' brief self introduction</td>
</tr>
<tr>
<td>09:20 – 09:35</td>
<td>Brief on the main results of the First OA Workshop  &lt;br&gt; <em>Mr Wenxi Zhu</em></td>
</tr>
<tr>
<td>09:35 – 09:50</td>
<td>Conduct of the workshop  &lt;br&gt; <em>Dr Somkiat Khokiattiwong, Dr Rusty Brainard</em></td>
</tr>
<tr>
<td>09:50 – 17:30</td>
<td>Lecture Session  &lt;br&gt; 09:50 – 10:45 Introduction to CO₂ Chemistry in seawater (Part 1) &lt;br&gt; <em>Dr Andrew Gilmore Dickson</em>  &lt;br&gt; 10:45 – 11:00 Group Photo and Coffee Break  &lt;br&gt; 11:00 – 12:00 Introduction to CO₂ Chemistry in seawater (Part 2) &lt;br&gt; <em>Dr Andrew Gilmore Dickson</em>  &lt;br&gt; 12:00 – 13:00 Lunch  &lt;br&gt; 13:00 – 13:30 Introduction on how to use CO₂SYS &lt;br&gt; <em>Dr Andrew Gilmore Dickson</em>  &lt;br&gt; 13:30 – 14:15 Introduction to monitoring of biological parameters &lt;br&gt; <em>Dr Rusty Brainard</em>  &lt;br&gt; 14:15 – 15:00 Monitoring bioerosion &lt;br&gt; <em>Dr Ian C. Enochs</em></td>
</tr>
</tbody>
</table>
15:00 – 15:15 Coffee Break
15:15 – 16:00 Monitoring accretion and calcification
   Dr Thomas A. Oliver
16:00 – 16:45 Monitoring biodiversity
   Dr Rusty Brainard
16:45 – 17:30 Globally coordinated ocean acidification observation and IOC-UNESCO’s role in GOA-ON
   Dr Kirsten Isensee
18:00 Welcome Reception
   hosted by the Phuket Marine Biological Center (PMBC)

Thursday 27 August 2015

08:30 Gathering at the lobby of the Kantary Bay Hotel, Phuket, and departure for the Phuket Marine Biological Center (PMBC)
   Venue: Meeting Room, on the second floor of the Phuket Aquarium, Phuket Marine Biological Center (PMBC)
09:00 – 12:00 Presentations of existing OA monitoring by participants
12:00 – 13:00 Lunch
13:00 – 17:30 Break-out session
   Moderator: Dr Rusty Brainard, Dr Suchana Chavanich, Dr Andrew Gilmore Dickson, Dr Somkiat Khokiattiwong
   Discuss and prepare realistic and achievable Standard Operating Procedures (SOPs) for monitoring carbonate chemistry and biological parameters for each pilot site for each country/institution
17:30 – 18:00 Plenary closing review of day

Friday 28 August 2015

08:30 Gathering at the lobby of the Kantary Bay Hotel, Phuket, and departure for the Phuket Marine Biological Center (PMBC)
   Venue: Meeting Room, on the second floor of the Phuket Aquarium, Phuket Marine Biological Center (PMBC)
09:00 - 10:30 National development and consultation on SOPs for implementation of OA monitoring for pilot sites.
10:30 – 10:45 Coffee Break
10:45 – 12:00 Summarize national SOPs and work plans
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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</thead>
<tbody>
<tr>
<td>12:00 – 13:00</td>
<td>Lunch</td>
</tr>
<tr>
<td>13:00 – 17:30</td>
<td>Discussion on next steps toward implementation of OA monitoring in the region</td>
</tr>
<tr>
<td>18:00</td>
<td>Farewell dinner</td>
</tr>
<tr>
<td></td>
<td><em>hosted by the IOC Sub-Commission for the Western Pacific (WESTPAC)</em></td>
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</table>
Technical Note for Participants
Technical Note for Participants

This workshop, as an follow up to the first one on Research and Monitoring of the Ecological Impacts of Ocean Acidification (OA) on Coral Reef Ecosystems (Phuket, Thailand, 19-21 January 2015), aims to build/enhance the OA monitoring capacity at the pilot sites identified by each of the countries during the first OA workshop. To this end, all nominated participants are required to:


ii. Since all participants will be divided on 27 August into two groups respectively on biology and carbonate chemistry. Each participant will be invited to brief on their existing monitoring capacity within their site(s). (Note: participants could also select other sites than these listed from the first OA workshop.) Resource Persons will assist/advise to improve if needed. Then two persons from each country shall meet to come up with a comprehensive monitoring plan for their site(s); On 28 August all will meet plenary to present their comprehensive monitoring plan.

When preparing your talk, please keep in mind the following inexhaustible list of questions:

- What minimum physical, chemical and biological parameters were measured? Where? At what depths?
- What is the desired spatial (what depths? how many?) and temporal resolution (frequency) of these measurements?
- What (parameters) and where are the gaps in present observing systems? Where and what new measurements do we need?

Important links:

- **Online OA short course:**
  http://www.whoi.edu/page.do?pid=33598
  https://www.youtube.com/watch?v=dR917nXLEHU
  https://www.youtube.com/watch?v=yhwc9UnNHHY

- **Guide to Best Practices for Ocean CO₂ Measurements (standard methods for OA water sampling and analysis):**

- **Guide to best practices for ocean acidification research and data reporting:**

- **Certified Reference Material:**
  http://scrippsscholars.ucsd.edu/adickson/biocv

- **CO₂ sys for calculating the carbon system:**
  http://cdiac.ornl.gov/ftp/co2sys/
- Existing OA observational programs for ideas and potential collaborations:
  http://www.goa-on.org/

- Relevant Documents:
  - DISSOLVED INORGANIC CARBON (DIC) AND TOTAL ALKALINITY (TA) SAMPLING: PLANNING AND SAMPLE COLLECTION
  - AUTONOMOUS REEF MONITORING STRUCTURES (ARMS)
    - Overview
    - Assembly
    - Deployment
    - Recovery
    - Processing
Related Standard Operating Procedures (SOPs)
3.1 DISSOLVED INORGANIC CARBON (DIC) AND TOTAL ALKALINITY (TA) SAMPLING: PLANNING AND SAMPLE COLLECTION

Section 1: Considerations in planning your field sampling

Sample bottle type and size—In order to run both DIC and TA analyses on the same sample, a 500mL sample is needed. If only one of the two analyses is needed (DIC or TA), then a 250 - 300mL sample is sufficient. In order to calculate all the parameters of the carbon system (pCO2, pH, carbonate species, saturation states (Ω), etc.) both DIC and TA must be measured. Sample bottles must be of a certain type and quality in order to ensure no gas exchange or reactivity/changes in alkalinity occurs. CRED uses Corning PYREX reagent bottles with PYREX stoppers (Fisher Scientific USA, http://www1.fishersci.com/ecomm/servlet/fsproductdetail?position=content&tab=Items&productld=765795&fromSearch=0&catlogld=29104&storeid=10652&langId=-1).

Water sampler size—In order to collect your seawater samples properly, you will need a water sampler that can collect several times the volume of seawater needed to completely fill the sample bottles, in addition to the volume needed for any other samples to be collected from the same sampler. This is because the collection method of DIC and TA involves overflowing the sample bottle with three full exchanges of seawater to collect a clean sample. Also the DIC and TA seawater sample must not include the last water to come out of the sampler, which will have been exposed to air as the sampler is emptied into the sample bottle, resulting in a contaminated sample (DIC concentration measured from a sample is a function of CO2 gas exchange, therefore collecting a seawater sample that has been in contact with the atmosphere results in an inaccurate measurement). CRED uses a 5 L Niskin bottle (General Oceanics, http://www.generaloceanics.com/home.php?cat=9) to collect a 500 mL sample, which provides ample volume to collect for other analytes as well.

Section 2: Preparation prior to drawing the DIC/TA samples

Prepare a saturated solution of mercuric chloride (HgCl2)—Read the Material Safety Data Sheet (MSDS) for mercuric chloride (USA, http://www.scientelab.com/msds.php?msdsslid=9924616). HgCl2 is a hazardous material and should be treated as such in the laboratory and for sample transportation. The U.S. Department of Transportation allows exceptions from the Hazardous Materials Regulations (HMR; 49 CFR parts 171-180) concerning the shipment of water samples containing limited quantities of various Class 8 corrosive materials, of which HgCl2 is included. HgCl2 can be shipped in water solutions at concentrations of 0.004% by weight or less. The saturation of mercuric chloride is 7.4 g per 100 mL of deionized water (DIW) - this is the minimum ratio. CRED suggests a 1:10 ratio for a saturated HgCl2 solution; e.g. 10 g mercuric chloride salt per 100 mL DIW. Standard volumes used for saturated HgCl2 solutions are 0.02-0.05% of the total sample volume. CRED injects 200 µL of saturated HgCl2 solution into a 500 mL seawater sample using a fixed volume pipette (Fisher Scientific USA, http://www1.fishersci.com/ecomm/servlet/itemdetail?storeId=10652&langId=-1&catalogId=29104&productld=2353925&distyoe=0&highlightProductsItemsFlag=Y&fromSearch=1&searchType=PROD&hasPromo=0). Ensure that your pipette is capable of dispensing 200 µL properly. Make sure that the HgCl2 solution stays saturated at all times by checking to see that HgCl2 salt crystals remain in the bottom of the bottle. Note: if taking DIC and TA samples separately, both samples must be poisoned with HgCl2.
Label the sample bottles in at least two locations—Bottle numbers can rub off, which could result in loss of data for any samples that cannot be matched to the field sampling log. Each bottle should be labeled with a unique number on the shoulder of the bottle, reducing the possibility of the label being rubbed off. CRED uses DecoColor paint markers for labeling the sample bottles, as labels written with Sharpies (and similar markers) can rub off. Do not apply adhesive paper labels to the bottles, as they are problematic when sample bottles get wet in the field and when cleaning the bottles post DIC/TA analysis.

Grease the sample bottle stoppers—Apply 4-5 thin vertical strips of Apiezon L ultra-high vacuum grease around each bottle’s stopper. After sampling, insert a greased stopper into the neck of the sample bottle, seat the stopper, and twist to spread the grease evenly and form an airtight seal.

Sampling noodle—Soak sampling tubing (noodle) in a bucket of clean water before the first sample and between subsequent sample helps to prevent bubbles from forming in the noodle during sampling. Note: bubbles in the sampling tubing (noodle) during sample collection are not acceptable and compromise the quality dissolved gas samples. Noodles may be either a single piece of Tygon tubing or consist of a narrower piece of flexible tubing inserted into a larger diameter piece of rigid tubing, and held in place with a cable tie. For single piece noodles, we recommend marking the noodle so that the same end is placed on the water sampler each time. For two piece noodles, the end of tubing used for contact with the Niskin bottle nipple will be obvious.

Prepare your log sheets—Prepare log sheets with all necessary information about locations and depths to be sampled, geographic coordinates, bottle numbers, etc. Remember to account for supporting information that accompanies your samples (The most accurate carbonate system calculations require salinity, temperature, pressure or depth, and phosphate and silicate concentrations).

Section 3: DIC/TA sample drawing procedure

Draw samples immediately after Niskin bottles come aboard—Dissolved gases must be sampled before other, less sensitive samples such as nutrients and salinity are collected. If multiple gas collections are to be made from the same bottle, the correct order of sampling is oxygen first, and then dissolved inorganic carbon parameters (pCO₂, pH, dissolved inorganic carbon, and alkalinity).

Check the Niskin bottle for leaks—Before sample collection, make sure sample water is not leaking from around the end caps, air valve or stopcock/petcock. If the end caps did not seat correctly or valves were not properly closed, the sample is compromised. Check that the tubing between the end caps is in good condition and attached correctly. If no leaks are observed, collect the seawater sample.

Fill the sample bottle—Attach the designated end of the Tygon tubing (noodle) to the stopcock/petcock nipple of the Niskin bottle sampler. After rinsing the Tygon noodle prior to sample collection, insert the noodle into the bottom of the sample bottle, open air valve at the top of the Niskin, open water valve (stopcock/petcock), and begin water flow. Invert the sample bottle over the tube to rinse the inside of the bottle carefully with the sample water, eliminating any air bubbles which may have stuck onto the sample bottle’s walls. It is critical to sample accuracy to prevent exposure of the sample to ambient atmospheric air bubbles. Slowly right the bottle and keeping the noodle at the bottom of the sample bottle, begin to fill, pinching the tubing (if necessary) to control the influx of sample. Allow the
bottle to fill completely and to overflow 3 full volumes (count how long it takes for the bottle to initially overflow (e.g. 20 sec), and then allow it to continue to overflow for 2x this amount of time (e.g. 40 additional sec) for a total of 60 sec of overflow). Pinch the noodle to stop the sample water flow while the tubing is still touching the bottom of the sample bottle. Then withdraw the noodle while continuing to pinch the noodle and restrict the flow of sample water from the Niskin. The volume displaced by the noodle establishes a "calibrated headspace of ~1% of the total sample bottle volume, which allows for possible sample expansion during transportation to the laboratory. Once the sample bottle is full and "calibrated," check the bottle walls for any bubbles. If you see any bubbles inside the sample bottle, discard the sample and redraw.

**Carefully add 200 µL of HgCl2**—Using a pipette, inject HgCl₂ solution into the 500 mL sample bottle (100 µL for 250–300 mL bottles). Do not submerge pipette tip into sample. Do not try to remove pipette tip from within sample bottle if the pipette tip is ejected by accident (DIC/TA analyses can still be conducted if pipette tip is in sample bottle).

**Insert greased stopper into neck of bottle and twist**—To form a good airtight seal between the stopper and bottle. If there are horizontal streaks visible in the greased seal, then use more grease on future samples.

**Seal the sample bottle with rubber band and collar**—As in pictures below. Securing the stopper is critical for proper storage, so if a band is lost, or breaks, use electrical tape over the stopper and under the bottle.

- Place the plastic collar through the middle of the rubber band (panel A).
- Pull both sides of the rubber band through the middle of the collar (panel B).
- While holding the collar, pull the rubber band down over the stopper and pinch the collar tightly around the neck of the bottle (panel C). Be sure to pull the collar down so that it is below the neck of the bottle. This sample (panel D) has the correct volume of headspace and a properly fit clip and rubber band.

**Invert the sample several times to mix the mercuric chloride thoroughly.**

**Dip each bottle in a bucket of clean fresh water**—Only up to the neck, towel dry, and place in the sample bottle storage crate. Bottles can be stored at room temperature but should be kept out of direct sunlight or high temperature. If cool/air conditioned storage is available, that is preferable. Samples should never be frozen.

**Take duplicate samples as you see fit**—e.g. 10% of the total number of samples. Consider taking duplicates at all critical sampling sites.
Section 4: Inorganic carbon chemistry

From the DIC and TA analytical measurements, you can calculate all remaining inorganic carbonate system parameters—$p$H, pCO$_2$, fCO$_2$, aragonite and calcite saturation states ($\Omega_{\text{arag}}$, $\Omega_{\text{calc}}$), and the concentrations of bicarbonate ion ([HCO$_3^-$]), carbonate ion ([CO$_3^{2-}$]), and dissolved carbon dioxide ([CO$_2$])—using a program called CO2SYS. To do so, we require:

- **Water temperature ($T$)** at the time of sample collection.
- **Pressure ($P$)** that the seawater sample was collected (the depth at which the Niskin bottle fired to capture the sample).
- **Salinity ($S$)**—It is possible that the analytical laboratory conducting the DIC and TA analyses will measure salinity of all samples using a thermosalinograph integrated into their analytical system, but high quality salinity measurements (from bottle analyses (highest quality) or CTD measurements) are best.
- **Nutrients**—To make the highest quality carbonate system calculations, phosphate ([PO$_4^{3-}$]) and silicate ([SiO$_4^{4-}$]) concentrations for each sample are required. Not having nutrient data can introduce an error on the order of 1–5%, possibly higher, depending on nutrient concentration, in the resulting calculated pCO$_2$ values.
3.2 AUTONOMOUS REEF MONITORING STRUCTURES (ARMS)

3.2.1 Autonomous Reef Monitoring Structures (ARMS) Overview

Autonomous Reef Monitoring Structures (ARMS) are small, long-term collecting devices designed to mimic the structural complexity of a coral reef and attract colonizing invertebrates. Developed by CRED in partnership with the Census of Marine Life (CoML), Census of Coral Reef Ecosystems (CReefs), ARMS were created to assess and explain the diversity, distribution, abundance, and community structure of the cryptofauna community (the most diverse community of organisms on a coral reef) on a global scale.

An ARMS unit is essentially a tier of nine 23 cm x 23 cm gray, Type I PVC plates stacked in an alternating series of open and obstructed formats attached to a 35 cm x 45 cm base plate. The entire structure is affixed to the sea floor and remains on the benthos for 1-3 years during which time it becomes colonized with marine organisms.

**ARMS provide a systematic, consistent, and comparable method to monitor cryptofauna diversity.**

Upon recovery, the ARMS unit's 8 layer tier is encapsulated within a mesh-lined container to prevent the escape of motile (moving) organisms. The units are disassembled plate by plate, with both sides photo-documented for spatial analyses of sessile (non-moving) organism coverage. 2 mm, 500 µm, and 100 µm geologic sieves are used to obtain three motile size fractions. The largest fraction (> 2 mm) is sorted into morphotaxonomic groups and can be processed via standard voucher-based molecular barcoding techniques. The two smaller motile fractions are processed via metabarcoding next-generation sequencing techniques. The sessile organisms are scraped off the plates, homogenized, and preserved for metabarcoding next-generation sequencing.
A typical ARMS unit yields hundreds of specimens of motile and sessile invertebrates from many tens of species thereby making them an ideal platform to examine biodiversity in a standard, replicable, and systematic manner. Preliminary estimates of the number of unique DNA sequences (i.e. species) from the metagenomic metabarcoding analyses is >1000 for an individual ARMS unit on a coral reef. They improve our ability to monitor, measure, and relate the cryptofauna community with ecosystem processes and provide baseline data across large biogeographic, environmental, human impact, and management protection gradients. They have been adopted as a key biodiversity assessment tool by NOAA's National Coral Reef Monitoring Program (NCRMP) and Ocean Acidification Program's climate monitoring stations in the Pacific. They are also a central component of the Smithsonian's Global Marine Biodiversity Project.

The ARMS Project has expanded on a global scale

Locations of current ARMS deployments

More than 600 ARMS have been deployed throughout the Pacific, Indian, and Atlantic Oceans

- 2006-2008 — Original design and testing of ARMS prototypes. The first ARMS units were deployed at French Frigate Shoals during the CReefs research cruise
- 2008-present — CRED incorporated ARMS into their Pacific Reef Assessment and Monitoring Program (Pacific RAMP) research cruises to:
  - American Samoa
  - Northwestern Hawaiian Islands
  - Main Hawaiian Islands
  - Guam
  - The Commonwealth of the Northern Mariana Islands (CNMI)
  - Pacific Remote Island Areas
- 2007-2009 — CReefs Australia deployed ARMS at Lizard and Heron Islands (GBR) and Ningaloo Reef
- 2009 — CRED collaborated with partners on expansion to the following locations:
  - Western Indian Ocean: Reunion, Europa, and Glorieuses Islands
- 2010 — CRED collaborated with partners on expansion to the following locations:
  - Grand Cayman Islands
  - Puerto Rico
  - Sangihe Island in North Sulawesi Indonesia
  - Bali, Indonesia

- 2012 — CRED collaborated with partners on expansion to the following locations:
  - Philippines - Verde Island Passage and Tubbataha Reefs
  - Dry Tortugas with NOAA's Florida Keys National Marine Sanctuary
  - Florida Keys with NOAA's Florida Keys National Marine Sanctuary
  - Timor Leste with U.S. Agency for International Development (USAID) and Conservation International
  - Puget Sound, Washington with Northwest Fisheries Science Center
  - Chagos Archipelago with James Cook University

- 2012 — Smithsonian Institution expanded to the following locations:
  - Belize
  - Jordan
  - Florida (Indian River)
  - Chesapeake Bay (Washapreague)
  - Panama (Bocas)
  - Taiwan
  - Curacao

- 2012 — Australian Institute of Marine Science deployed ARMS along CO2 vents in Papua New Guinea

- 2012 — CRED collaborated with the European Union’s DEVOTES (DEVelopment Of innovative Tools for understanding marine biodiversity and assessing good Environmental Status) Project to expand ARMS to the four regional European Seas

- 2012 — Recovered ARMS from Kimbe Bay, Papua New Guinea

- 2012 — Smithsonian Institution deployed ARMS in Saudi Arabia

- 2013 — ARMS were incorporated into the NOAA National Coral Reef Monitoring Program's and Ocean and Acidification Program's climate and ocean change monitoring stations

- 2013-2017 — CRED is collaborating with University of California, Los Angeles (UCLA), Smithsonian Institution, Moss Landing Marine Laboratories, and San Diego State University (SDSU) through a National Science Foundation PIRE (Partnerships for International Research and Education) grant to deploy ARMS throughout Indonesia

- 2014 — ARMS recovered from 8 sites on north coast of Timor Leste

- 2015 — ARMS recovered by CRED and PIRE grant partners from Verde Island Passage and Tubbataha Reefs, Philippines

- 2015 — NOAA’s Northwest Fisheries Science Center with cooperation from Shannon Point Marine Center (Western Washington University deployed ARMS in the southern Salish Sea
The ARMS Project is Multi-Institutional

- Australian Government
- Australian Institute of Marine Science
- NOAA
- National History Museum
- JIMAR
- University of New South Wales
- Smithsonian Institution

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3.2.2 Autonomous Reef Monitoring Structures (ARMS) Assembly

ARMS Assembly
- Materials
- Assembly Schematic
- Assembly Procedures
  - Step 1: Top Plate
  - Step 2: Open Layer
  - Step 3: Closed Layer
  - Step 4: Additional Layers
  - Step 5: Completing the ARMS Tower
  - Step 6: Base Plate Assembly
  - Completed ARMS

Materials

<table>
<thead>
<tr>
<th>QTY</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>PVC Layer Plates (225mm x 225mm x 6.3mm)</td>
</tr>
<tr>
<td>1</td>
<td>PVC Base Plate (450mm x 350mm x 12.7mm)</td>
</tr>
<tr>
<td>8</td>
<td>PVC Short Cross Spacers (140mm x 20mm x 12.7mm)</td>
</tr>
<tr>
<td>4</td>
<td>PVC Long Cross Spacers (300mm x 20mm x 12.7mm)</td>
</tr>
<tr>
<td>16</td>
<td>1/2&quot;-tall Nylon Spacers for 1/4&quot; Bolts (0.257&quot; ID x 0.500&quot; OD x 0.500&quot;)</td>
</tr>
<tr>
<td>4</td>
<td>1/4&quot;-20 x 8.5&quot; Bolts, Stainless Steel</td>
</tr>
<tr>
<td>12</td>
<td>1/4&quot; Flat Washers, Stainless Steel (two different sizes pictured above as larger washers were used for the baseplate)</td>
</tr>
<tr>
<td>8</td>
<td>1/4&quot;-20 Jam Nuts, Stainless Steel</td>
</tr>
<tr>
<td>1</td>
<td>Tube of Aqualube/Aquashield™ or Silicone Grease (optional but highly advisable)</td>
</tr>
</tbody>
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Assembly Schematic

Exploded view of an ARMS assembly (left) and views of a completed ARMS assembly (right top and bottom).
Assembly Procedures

Step 1: Top Plate
Place a washer on each of four bolts, and then run the bolts through the four corners of a single layer plate. This plate will now be the top plate of the ARMS under construction.

Step 2: Open Layer
Turn the top plate over, so the bolt heads and washers are down and the bolts are sticking up through the plate. Begin constructing the various layers of the ARMS. Slide 1/2” nylon spacers onto each bolt then add a PVC plate.
Step 3: Closed Layer
Closed layers are created by using one Long PVC Cross Spacer and two Short PVC Cross Spacers. Slide a long spacer onto bolts located diagonally across the ARMS. Slide the short spacer on the remaining bolts such that they each contact the long spacer at a right angle, thus creating 4 equally sized triangular spaces on the plate. Finally, add another layer plate to complete this first closed layer.
**Step 4: Additional Layers**
Continue to alternate construction of open layers and closed layers, until there are a total of four open and four closed layers.

**Step 5: Completing the ARMS Tower**
Once the last layer plate is added, thus completing the final closed layer, add a flat washer and jam nut to each of the four bolts and tighten each nut securely. (Note: It is highly advisable to add Aqualube/Aquashield™ or silicone grease to the threads of each bolt to prevent nuts from freezing to the bolts as a result of deployment in saltwater. This will greatly facilitate subsequent removal of the nuts during ARMS processing).
Step 6: Base Plate Assembly
The final step in ARMS construction is to attach the base plate to the tower of layers. Simply lay the base plate over the bolts (with the counter sunk side down) and add a washer and a lock nut to each bolt. Tighten securely.
**Completed ARMS**
With the base plate attached, the ARMS is now fully assembled.
3.2.3 Autonomous Reef Monitoring Structures (ARMS) Deployment

ARMS Deployment
- Deployment Equipment
- Weight Attachment
- Deployment
  - Lowering the Equipment
  - Installation
  - Alternative Installation Methods
  - Photography
  - GPS

**Deployment Equipment**

<table>
<thead>
<tr>
<th>QTY</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>1 (3)*</td>
<td>ARMS with base plate attached</td>
</tr>
<tr>
<td>4 (12)*</td>
<td>Heavy duty 24&quot; cable ties</td>
</tr>
<tr>
<td>4 (12)*</td>
<td>Heavy duty 36&quot; cable ties</td>
</tr>
<tr>
<td>2 (6)*</td>
<td>Weights (capped PVC pipe with lead inside)**</td>
</tr>
<tr>
<td>4 (12)*</td>
<td>3/8&quot; x 24&quot; stainless steel threaded rods with a chisel point on one end***</td>
</tr>
<tr>
<td>1</td>
<td>Sledge hammer per diver</td>
</tr>
<tr>
<td>1</td>
<td>Gear bag</td>
</tr>
<tr>
<td>1</td>
<td>Drop line with buoy</td>
</tr>
<tr>
<td>1</td>
<td>Camera</td>
</tr>
<tr>
<td>1</td>
<td>GPS</td>
</tr>
</tbody>
</table>

*Number in parentheses is the quantity required for one survey site of 3 ARMS.
**This is optional but helps in high wave energy environments and overall in stabilizing the ARMS units.
***Bring extra stainless steel threaded rods (if a rod is bent during installation, it must be replaced with a new rod). There are other installation methods that range from drilling eye-bolts and using cables or zip ties to connect the unit to heavy anchors. You can improvise based on budget, wave exposure, and habitat.
Weight Attachment

One method to help stabilize the ARMS unit on the benthos is to add weights to the device. Prior to deployment, you can attach a PVC pipe weight to each handle using two 24” zip ties per side. It is best to thread each 24” zip tie through the handle space twice such that each zip tie forms two loops around the PVC weight. Ensure that the zip ties are tightened around each end of the PVC pipe weight so that the weights are securely attached to the ARMS.
Deployment

NOAA deploys ARMS in sets of three on hard bottom forereef habitats at depths of 10-15 meters. Individual ARMS units within a survey site are placed 2-4 meters apart.

Lowering the Equipment

In order to minimize adverse impacts to the coral reef habitat, we lower all of the ARMS equipment to the bottom using the buoyed drop line. The bitter end of the line is run through one handle of each of the 3 ARMS and clipped back to itself. The tool bag is clipped to the line just above the ARMS.

A free-diver briefly surveys the desired survey area for an appropriate sandy / rubble location into which the equipment can be lowered from the support boat. Once the free diver finds a suitable location, hand signals and/or voice commands are used to direct the support vessel over the appropriate location in which to lower the equipment.

Once the equipment is lowered, the surface buoy attached to the drop line marks the dive location. Divers need only follow the drop line down to the equipment to begin ARMS installation.

Figure 3: ARMS equipment ready for lowering off of the small boat.

Figure 4: ARMS being lowered off of the small boat (left). Figure 5: ARMS lowered into a rubble patch (right).
Installation

Figure 6: Using a sledgehammer to pound in the stakes.

Figure 7: Zip tying the stakes to the ARMS unit.

Divers look for rubble or bare patches of substrate in which to install the ARMS so as to minimize any collateral damage to the coral reef habitat. ARMS are installed approximately 2 to 5 meters apart as the topography allows.

If installing ARMS units with stainless steel shanks, drive a stake using a sledge hammer through each corner hole of the base plate. If possible, stakes should be installed perpendicular to the substrate to facilitate ARMS removal at a later date by simply lifting it vertically off the stakes.

However, sometimes it is not possible to achieve a perpendicular orientation of the stakes. This is not a problem. Just be sure to hammer in the stakes to at least half of their length in whatever orientation allows this. Stability of the ARMS base plate is ultimately more important than stake orientation.

Once the stakes are driven into the substrate through the holes in the base plate, use the 36" heavy duty zip ties to secure the base plate to the stakes.

Thread a zip tie through a corner of the base plate and take multiple wraps around the stake before securing. Repeat for the remaining corners. A correctly installed ARMS unit should feel securely attached to the substrate with very little play (lateral or vertical) when manipulated by the diver.
If for some reason the stakes cannot be installed through the corner holes of the base plate, they may be installed through the handles at opposing angles (crossed). Similarly, use the 36" heavy duty zip ties to secure the base plate to the stakes.

**Alternative Installation Methods**

In hard bottom habitats, such as basalt, that are challenging to pound in stakes, you can alternatively use heavier weights to secure the ARMS unit to the benthos or a pneumatic drill to install eye bolts. Zip ties would be used to secure the weights to the base plate (see below) and in drilling, zip ties secure the base plate to the eye bolts.

ARMS units secured to the benthos using weights. Long weights shown are 70 lbs each and the shorter weights are 40 lbs each.

**Photography**

Document the site with photos of the surrounding habitat as well as the deployed ARMS. If there is a particular sponge, algae, tunicate, bryozoans etc that is prevalent at the site, take a close-up image to document.

**GPS**

Mark a GPS point of the site. Make sure to swim over the spot and get the point directly above the ARMS.
3.2.4 Autonomous Reef Monitoring Structures (ARMS) Recovery

**Equipment for Dive**

- Something to encapsulate ARMS tier such as a milk crate lined with a 100 µm mesh or a square 18 Q container (a recovery crate)
- Knife or shears
- Tool bag
- Gear bag with buoyed line attached
- Floating line with a buoy attached to one end
- 2 ratchet straps, heavy-duty bungee cords, or cam straps per ARMS unit

*Note the number of recovery crates and buoyed lines brought down should equal the number of ARMS being removed that that site.

**Locating the ARMS**

ARMS may be challenging to recognize underwater after they've soaked for a period of time. To maximize dive time, it is suggested to first snorkel and free dive around the marked GPS position to find their location. This is especially important if ARMS are being redeployed at the same site. It prevents the divers from having to move the heavy deployment equipment underwater if the gear was lowered on a GPS point that was slightly off site.

**Recovery Procedures**

1. Bring recovery crate, tool bag with shears and knives, two ratchet straps or heavy-duty bungee cords, and an extra bag to hold weights, zip ties and stakes after removal. You will also need line(s) longer than the depth to use as a hand-tended recovery line or a lift bag to bring the ARMS unit and weights and stakes to the surface.

2. Using a knife or shears, remove the zip ties around the weights.
3. Remove the zip ties attaching the baseplate to the stakes.

4. When the ARMS unit is free of zip ties and weights, place the recovery crate over the ARMS unit.

5. Insert ratchet straps through the hand holes on the base plate and back through itself.
6. Pull or crank the latching straps until the cover is tightly sealed on the ARMS baseplate. You can use bungee cords as long as the tension is high enough to keep a good seal during the rest of the recovery process.

7. If you are not redeploying a new set of ARMS, remove the stakes and place them along with the weights and zip ties into the gear bag. If you are redeploying ARMS, lift the covered ARMS unit over the stakes and redeploy the new ARMS unit over existing stakes and proceed with installation.

8. Attach a buoyed line to the ARMS unit for surface recovery. **Note: experienced divers could carry an ARMS unit to the surface during their accent and not attach a buoyed line.
9. Once back in the small boat, fill up containers full of sea water to place the ARMS into.

10. Pull up the ARMS units attached to the buoyed lines individually.

11. Lift the ARMS out of the water and immediately place each ARMS unit in a tub full of seawater with a battery operated aquarium bubbler.
If your recovery crate is not mesh lined such as the 18Q container pictured below, remove the crate from the tier in a tub of seawater and add bubblers.

12. Transport the recovered ARMS to ship or shore station for further processing as soon as possible.
3.2.5 Autonomous Reef Monitoring Structures (ARMS) Processing

**Transfer and Disassembly**

Transfer the recovered and intact ARMS unit into a new container (a disassembly tub) containing seawater and air pumps for oxygenation. Water from the used recovery container will not be sieved for processing and thus can be discarded. The holding crate used for retrieval is still attached to the ARMS unit.

If the recovery crate was removed in the field, then you must transfer the recovery container with the ARMS unit and the existing seawater to your processing location. The field recovery container will then become your disassembly tub and you can start dissembling the ARMS unit as explained below.

**ARMS Processing**

- Transfer and Disassembly
- Photography
- Brushing
- Sieving
- Sorting and Documentation
- Scraping
- Processing Products

Detach the latching straps and carefully lift off the holding crate. Dip and shake the crate in the seawater to free organisms trapped within the mesh lining and corners. This may require flushing the liner with seawater over the tub or using forceps to pick off unrelenting critters.
Once the recovery crate is removed, start disassembling the ARMS unit. Remove the nuts attaching the base plate to the ARMS tier using a 7/16" wrench or drill with a 7/16" socket. Once removed, lightly brush the base plate in the tub with a paint brush to remove any motile organisms. When this is complete, the base plate may be discarded. Proceed to remove the next set of nuts that is keeping the tier intact using the 7/16" wrench or drill. Once the nuts are all removed, the layers are accessible and ready for processing.

Photography

Remove the bottom layer of the ARMS unit (which is plate 9) and rinse it lightly in the container by dipping it side to side in the disassembly tub. This will help remove any sand particles on the plate thereby providing a cleaner surface for imaging the sessile organisms. Place the rinsed layer in a photo tray and include the appropriate label. Our labels contain the first three letters of the removal location followed by the site number, year, the ARMS ID letter, plate #, and a T or B to indicate top or bottom (e.g. OAU_01_2010_ARMS_A_4_T (Island of Oahu, Site 01, Year 2010, ARMS A, Plate 4, and Top of Plate)).

Take an initial photo of the plate with the label. Then photograph the whole plate without the label. Take a close up image of each quarter of the plate, the center, and of anything of interest. Turn the plate over and repeat. Take the highest quality JPEG or RAW image at the highest resolution, ensuring that enough memory is available to handle the file size. Images can be used for analyses of sessile recruitment and composition.
Alternatively you can use a tripod and set the camera to the following manual settings:

- Shutter speed - 200s
- F-stop - F11
- ISO - 400

When using the tripod at these settings, take one photo of the plate with the label and several images without the label. Make sure to avoid any artificial sunlight or shadowing from hitting the plate.

We use the following camera set up:

- Nikon D90
- AF Micro-Nikkor 60 mm lens 1:2.8 D
- Nikon SU-800 speedlight
- 2 - Nikon SB-R200 wireless remote speedlights
- 2 - Joby Gorillapod SLR Zoom Tripods
- Manfrotto 055X Pro Tripod
- Manfrotto 808RC4 3-Way Panhead

**Brushing**

Once the top and bottom images have been taken, gently brush both sides of the plate using a standard paint brush into a new rinse bucket containing sea water to remove any non-sessile organisms that are still attached. Place the brushed plate into a fresh container with seawater and air bubblers if the plates are not immediately scraped (see below for scraping). Remove the next layer from the disassembly tub, photograph it, and repeat the series of steps described above.
Sieving

Once all of the plate layers in the ARMS unit have been photographed, the seawater from the disassembly tub, photo tray, and rinse bucket is sieved through adjoining 2 mm and 500 µm sieve pans and an attachable 100 µm mesh hand net or sieve. Place the material collected in the 500 µm sieve and 100 µm net into two separate jars. Fill jars with EtOH, 20% DMSO, or RNA later - and label accordingly. The preserved 500 and 100 µm sample fractions undergo mass sequencing techniques. The ≥ 2 mm size fraction can either be bulked preserved, like the 500 and 100 µm fractions, with the understanding that they will be sorted at a later date or sorted at the time of processing.

Sorting and Documentation

Sorting the ≥ 2 mm size fraction is more efficient immediately after processing because the organisms are alive, intact, and colorful. Ethanol as a preservative fades away specimen coloration, can separate annelid segments and can detach crustacean limbs when bulk preserved. Immediate processing of the ≥ 2 mm size fraction also provides you with the opportunity to photograph the specimens for vouchering. Regardless of the method you choose, make sure that
during the sorting process specimen labels are assigned appropriately. We label specimens using the first three letters of the island code and include a unique specimen number (for example OAH-001, OAH-002, OAH-003, etc.).

When photographing specimens, the first image should have the unique specimen label in the image. Subsequent images can be taken without the label for finer details. When images and identifications are complete, preserve the specimen(s) with their unique specimen ID label in a vial with ethanol, 20% DMSO, or RNA later if you plan on vouchering the specimen or subsampling for DNA barcoding.

Scraping

All plates (n = 9) from an individual ARMS unit are scrapped en masse and bulk preserved. Try to remove all of the encrusting sessile and bivalve organisms from each plate using a tool such as a paint scraper. Once all 9 plates have been scraped, we place all the scrapings into a blender and homogenize the contents for one plus minutes, adding sea water if necessary.
Next, we pour the blended scrapings into a 40 µm hand net and use sea water to remove all the contents from the blender into the hand net. We then carefully squeeze the hand net to drain the seawater, leaving the homogenized contents entrapped within the net. Seawater is added to the net and filtered through until the drained coloration of the seawater is no longer muddy looking. At that point, the content within the net is subsampled. Approximately 10 grams is placed in a 50 ml falcon tube and filled with 20% DMSO, ethanol or RNA later. This is repeated 3 times creating 3 replicate samples. These samples, like the 500 µm and 100 µm fractions, will eventually undergo mass sequencing techniques using a MoBio Soil PowerMax@Soil DNA Isolation Kit for DNA extraction. The remaining sample is placed in a whirlpak and frozen. Eventually the frozen material will be dried and weighed to calculate a biomass metric.
Processing Products

When processing is complete, you should have at least the following from one ARMS unit:

- High resolution plate imagery
- One jar of bulked 500 µm specimens
- One jar of bulked 100 µm specimens
- A minimum of three 50 ml falcon tubes containing 10 g of the homogenized scrapings in a preservative
- One whirlpak of frozen scrapings

If you do not immediately sort through the ≥ 2 mm size fraction, then you should also have:

- One jar of bulked ≥ 2 mm specimens.
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Information Note for Participants
Dear Participants,

Welcome to Phuket for the Second WESTPAC Training Workshop on “Research and Monitoring of the Ecological Impacts of Ocean Acidification on Coral Reef Ecosystems” which will be held from 26 to 28 August 2015 at the Phuket Marine Biological Center (PMBC), Phuket, Thailand. To facilitate your travel preparations, please find below the information on logistic arrangements.

1. **Workshop Venue**

The workshop will be held at

**Phuket Aquarium**  
Phuket Marine Biological Center (PMBC)  
51 Moo 8, Tambon Wichit, Mueang, Phuket 83000 Thailand  
Tel: (66) 76 391 126  
Fax: (66) 76 391 406/(66) 76 391 051  
Email: skhokiatiwong@gmail.com, varinthavasi@gmail.com  
Website: http://phuketaquarium.org/en

Location Maps are attached as **Annex A**.

2. **Hotel Accommodation**

To ensure the application of a special room rate set for participants to this workshop, PMBC will provide all participants with the reservation service.

Detailed Information on the hotel and special rate for the workshop could be found below.

**Kantary Bay Hotel (1.0 km from the workshop venue)**

**Address:** 31/11 Mu 8, Sakdidej Rd., Cape Panwa Phuket 83000 Thailand  
**Tel:** (66) 76 39 1514  
**Fax:** (66) 76 39 1208  
**Email:** meeting@capepanwa.com  
**Website:** [http://www.kantarybay-phuket.com/accom.html](http://www.kantarybay-phuket.com/accom.html)

<table>
<thead>
<tr>
<th>Room Type</th>
<th>Special Room Rate</th>
<th>No. of Room Guaranteed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studio Suite (Single/Twin)</td>
<td>THB1,400 (US$ 41.3)</td>
<td>30 rooms</td>
</tr>
</tbody>
</table>

The **special room rate** is inclusive of 10% service charge, VAT and breakfast.  
**Free WiFi** is available in-room and public areas of the hotel.  
**Check-in time:** 14:00 p.m.; **Check-out time:** 12:00 p.m.  
**Late Check-Out time:** 18.00 p.m., with extra 50% of room rate charged.
3. Transportation

The Phuket International Airport is located 45.6 kilometers (1 hr without traffic) away from the hotel and the workshop venue. Phuket Marine Biological Center (PMBC) will provide assistance on local transportation arrangement between the Phuket International Airport and the hotel.

IMPORTANT NOTE!

Please complete the attached Registration Form (Annex B), and send it, via email, to Ms Varinthavasi at varinthavasi@gmail.com with a copy to Ms Thapupsorn Hnoonim at t.hnoonim@unesco.org and provide her with required information to ensure either of the hotel room reservation and the transportation arrangement, preferably no later than 9 August 2015. Guideline for Transportation Arrangement is attached as Annex C.

4. Registration

Participants are required to register upon arrival at the workshop venue (Phuket Aquarium) on the morning of 26 August 2015. The registration desk for the Second WESTPAC Training Workshop on “Research and Monitoring of the Ecological Impacts of Ocean Acidification on Coral Reef Ecosystems” will open at 08:15 a.m., 26 August 2015, in front of the meeting room on the second floor of the Phuket Aquarium. Please provide your name card to the Secretariat upon registration.

5. Meal

International Buffet Lunch will be provided on all working days, from 26-28 August 2015 from 12:00-13:00 hrs. The place for lunch will be announced at the meeting.

6. Reception

Reception dinners are planned for the evening of 26 and 28 August 2015 for all participants from 18:00-20:30 hrs. The place for dinners will be announced at the meeting.

7. Visa Information

Participants requiring an entry visa to Thailand are strongly advised to apply for it with the Thai Embassy or Consulates in your country as soon as possible in order to secure the required entry visa prior to your departure.

Information on visa requirement and procedure can be found on the website of the Ministry of Foreign Affairs of the Kingdom of Thailand at http://www.mfa.go.th/main/en/services/123.

8. Weather, Time Zone and Currency

Temperature in Phuket in August ranges between 25°C to 32°C. There will be plenty of rain but usually falls in a short heavy bursts. In between, the weather is pleasant with comfortable temperatures and a cooling breeze. Participants may need to bring more casual clothing, mosquito lotion or spray if you deem necessary. Current weather conditions can be found at http://www.worldweather.org/en/city.html?cityId=579
The standard time zone in Thailand is GMT/UTC +7 hours. The currency in Thailand is Thai Baht (THB). The current exchange rate as at July 2015 is US$ 1 = THB 33.89. You can view exchange rates at www.xe.com. Participants are advised to exchange some Thai Baht (THB) prior to your departure, or upon your arrival at the Phuket International Airport. There are four currency exchange counters in the arrival hall and one counter in the departure hall.

9. Electricity

The electrical currents in Thailand are 220 volts with the following electrical outlets:

WESTPAC Office disclaims all responsibilities for medical, accident and travel insurances, for compensation for death or disability compensation, for loss of or damage to personal property and for any other losses that may be incurred during travel time or the period of participation. In this context, it is strongly recommended that participants will secure international medical, accident and travel insurances for the period of participation prior to departure.

Should you have any questions or require any assistance on the logistic arrangements, please feel free to contact:

**IOC Sub-Commission for the Western Pacific (WESTPAC)**
Ms. Thapupsorn Hnoonim
Tel: +66 2 141 1448; Fax: +66 2 143 9245
Cell phone: +66 81 528 8512
Email: t.hnoonim@unesco.org

Finally, we wish you a pleasant stay in Phuket, the Pearl of Andaman Sea!

IOC Sub-Commission for the Western Pacific (WESTPAC)
Annex A

Location Map (1)
Free shuttle services between the two hotels will be provided every fifteen minutes from 07:00 a.m. to 12:00 a.m.
Annex B

Registration Form

Second WESTPAC Training Workshop on Research and Monitoring of the Ecological Impacts of Ocean Acidification on Coral Reef Ecosystems

26-28 August 2015

Phuket Marine Biological Center, Phuket, Thailand

PERSONAL INFORMATION

Full Name (Dr./Mr./Mrs./Ms./Miss/Other):

______________________________________________________________________________

Company/Organization:

______________________________________________________________________________

Designation: _________________________________________________________________

Specialty/Occupation: __________________________________________________________

Mailing address: ___________________________ Country: _________________________

Postcode: _____________ Telephone: ______________ Fax: ________________________

E-mail address: _____________________________________________________________

☐ Do not allow my personal information to be listed in the proceeding

SPECIAL MEAL REQUEST

☐ Vegetarian

☐ Other:_______________________________________________________________
ACCOMMODATION

Please note that all the participants who received our sponsor will have to share a room with another person. The organizer will arrange a double room for two people in the same gender with breakfast.

Check in date: ________________________ Check out date: ________________________

ITINERARY: Flight Information

Arrival Date: ________________________ Airline: ________________________
Flight no: ________________________ Time: ________________________

Departure Date: ________________________ Airline: ________________________
Flight no: ________________________ Time: ________________________

NOTE:

TRANSPORTATION
The organizer will provide support for the airport transfer for the participants on the arrival date of August, 25th 2015 and the departure date of August, 29th 2015.

Signature________________________________________
(________________________________________)
Date__________________________________________

*Please complete Registration Form in English, and send it, via e-mail to:
Miss Varintha Vasinamekhim E-mail: varinthavasi@gmail.com with a copy to
Miss Thapupsorn Hnoonim E-mail: t.hnoonim@unesco.org
Telephone: 662 141 1448 Fax: 662 143 9245

Please return the completed Registration form, preferably no later than 9 August 2015
Annex C

Guideline for Transportation Arrangement

When you enter the Passenger Hall, please look for our representatives holding up a sign with “WESTPAC Ocean Acidification Training Workshop”. They will be standing in front of the exit from the customs checkpoint of International Arrival Hall.

In case of any problems due to unforeseeable circumstances, please call our mobile for assistance at 66 (0) 83 909 9732 (Ms Varintha) and/or 66 (0) 81 528 8512 (Ms Thapupsorn)