Update on Ocean Acidification work in the Philippines

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Current efforts

1. Baseline on carbonate parameters

2. Experiments - characterize physiological limits of coral species in response to stressors (temp, pH, nutrients)
pH and Aragonite Saturation – Baseline study

Are Philippine waters becoming acidic?
Map of sites with $\Omega$Ar and pH data in the Philippines as of 2015

- Bolinao, Pangasinan
- Benham Rise
- Twin Island, Batangas
- Lian, Batangas
- Puerto Galera, Mindoro
- Sablayan, Mindoro
- West Phil Sea
- Taytay, Palawan

Map of sites as of 2016

- Additional sites marked with green circles
Ocean Acid kit sent to “sites of opportunity”

Ocean Acid kit contains
5 bottles (250 ml)
Plastic bottle with HgCl₂
Waterproof notebook and pencil
Gloves and parafilm
Instructions
SAMPLING for OCEAN ACIDIFICATION parameters
(For sampling done by divers)

Materials:
- 5 pcs of 250-ml glass bottles
- Plastic bottle containing mercuric chloride solution
- Waterproof notebook and pencil
- Gloves

Procedure:
1. Collect samples from 3 sites:
   - Reef with good coral cover (from 1m below the surface and 1m above bottom)
   - Reef with poor coral cover (from 1m below the surface and 1m above bottom)
   - More offshore (from 1m below the surface)

2. If possible, rinse bottle first before filling. Gently fill each bottle to the top. Make sure there are no bubbles in the water collected or in the bottle walls. If there are bubbles, discard the sample and gently fill the bottle again.

3. Put 5 drops of mercuric chloride in each bottle with sample (wear gloves when handling mercuric chloride).
   - Cap the bottle tightly and shake. Make sure to re-tighten the cap a second time after an hour of filling the bottle. This is to ensure no sample leak and an airtight seal. Put parafilm around bottle lid.

4. Cap plastic bottle containing mercuric chloride solution tightly and keep in ziplock bag when not in use (mercuric chloride is hazardous material).

5. Store the bottles at room temperature out of direct sunlight or high temperature.

6. Please log in the notebook the following information:
   - Date and Time of collection
   - Sampling site and Description of site ((good coral cover, poor coral cover, offshore, etc)
   - GPS coordinates (lat and long)
   - Water depth and Sample depth (surface or bottom)
   - Sample bottle number

THANK YOU so much for helping us get baseline information on carbonate parameters (saturation state, pH) from different areas.

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Total Alkalinity Titrator (Kimoto)

Reference material for CO₂
Ω_{Ar} distribution in surface waters of the Philippines

<table>
<thead>
<tr>
<th>Ω_{Ar} Range</th>
<th>Basin Areas</th>
<th>Coastal Areas</th>
<th>Seagrass Areas</th>
<th>Mariculture Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-0.99</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>1.00-1.99</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2.00-2.99</td>
<td>27</td>
<td>18</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>3.00-3.99</td>
<td>27</td>
<td>122</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4.00-5.00</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Basin Areas
- Bicol Shelf: 2.76-3.26
- Benham Rise: 3.08-3.35
- West Palawan Shelf: 2.48-2.90

Coastal Areas: 2.71-4.09

Mariculture Area (Bolinao): 0.20-2.26
Seagrass Areas (Bolinao): 1.97-4.85
**pH** distribution in surface waters of the Philippines

<table>
<thead>
<tr>
<th>pH Range</th>
<th>Basin Area</th>
<th>Coastal Area</th>
<th>Seagrass Areas</th>
<th>Mariculture Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.40-7.59</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>7.60-7.79</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>7.80-7.99</td>
<td>3</td>
<td>19</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>8.00-8.19</td>
<td>51</td>
<td>118</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>8.20-8.39</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>&gt;8.40</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

**Basin Areas**
- **Bicol Shelf**: 8.041-8.129
- **Benham Rise**: 8.026-8.152
- **West Palawan Shelf**: 7.928-8.070

**Coastal Areas**: 7.793-8.199

**Mariculture Area (Bolinao)**: 7.418-7.999
**Seagrass Areas (Bolinao)**: 7.959-8.491
pH
Surface waters
Bolinao, Pangasinan
$Ω_{Ar}$
Surface waters
Bolinao, Pangasinan
Monthly $\Omega_{Ar}$ in Mariculture areas (Bolinao)
Monthly pH Distribution in Mariculture Area (Bolinao)
Study 1b. Characterizing physiological limits of selected coral species in response to environmental stressors

- Elevated temperature
- Lowered pH
- Increased nutrients

*Research Program on “Molecular genetic and genomic studies of coral resilience in support of coral restoration and rehabilitation efforts*
List of Candidate Coral species (Bolinao-Anda Reef Complex)

<table>
<thead>
<tr>
<th>Resistance to Bleaching</th>
<th>Growth Form</th>
<th>Coral Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fine Branching</td>
<td>Stylopora pistillata¹</td>
</tr>
<tr>
<td>2</td>
<td>Branching, Tabulate, Encrusting / Foliose</td>
<td>Seriatipora caliendrum²</td>
</tr>
<tr>
<td>3</td>
<td>Massive, Brain</td>
<td>Pocillopora damicornis²</td>
</tr>
<tr>
<td>4</td>
<td>Massive, Boulder</td>
<td>Acropora digitifera²</td>
</tr>
<tr>
<td>5</td>
<td>Various</td>
<td>Acropora tenius²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acropora millepora¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Montipora digitata¹</td>
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<tr>
<td></td>
<td></td>
<td>Acropora millepora¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isopora cuneata¹</td>
</tr>
<tr>
<td>1 = LOW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 = HIGH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 = LOW 
5 = HIGH


²Photo by Mikhael Clotilde S. Tanedo
³Photo by Tracy D. Tabalanza
Coral Larvae Response to Ocean Warming (T) and Ocean Acidification (OA)
Seawater pH adjusted to target pH via CO₂ dosing

Mass Flow Controller

Ambient pH: 8.1

OA(mid): pH 7.8

OA(high): pH 7.6

Control:
- pH 8.1, 27°C

OA(mid):
- pH 7.8, 27°C

OA(high):
- pH 7.6, 27°C

T(mid):
- pH 8.1, 30°C

T(mid-mid):
- pH 7.8, 30°C

T(mid-high):
- pH 7.6, 30°C

T(high):
- pH 8.1, 27°C

T(high-mid):
- pH 7.6, 30°C

T(high-high):
- pH 8.1, 27°C

Ambient temp: 27°C

T(mid):
- 30°C

T(high):
- 33°C

TEMPERATURE
CONTROLLED TANKS

TREATMENTS

METHODOLOGY

I. EXPERIMENT DESIGN (adapted from Baria et al., 2015)
II. LARVAL CULTURE (Edwards, 2010)

Fertilized eggs

Planulae larvae

Favites colemani

III. LARVAL RESPONSE to OCEAN ACIDIFICATION and WARMING

Planulae (sample size): 45 per treatment (15 per vial)

Survival Experiment

LARVAL SURVIVAL (%) (Baird et al., 2006; Nozawa and Okubo 2011)

LARVAL SIZE (µM) (Kareen)

Metamorphosis Experiment

LARVAL SURVIVAL (%) (Baird et al., 2006; Nozawa and Okubo 2011)

METAMORPHOSIS RATE (% day⁻¹) (Nakamura, et al., 2011)

10-DAY EXPOSURE to TREATMENTS

3-DAY EXPOSURE to TREATMENTS

METHODOLOGY
RESULTS and DISCUSSION

Larval survival and size after 10-day exposure to OA, T, and OAT

**Treatments**

- **Survival and larval size** was significantly affected by OAT.

- *Favites colemani* was relatively large (~300um).
Adult Coral Response to Anthropogenic Nutrient Stress (ANS), Ocean Warming (T), and Ocean Acidification (OA)

<table>
<thead>
<tr>
<th><strong>SURVIVAL (%)</strong></th>
<th>(Baird et al., 2006; Nozawa and Okubo 2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GROWTH RATE (% day⁻¹)</strong></td>
<td>Skeletal growth/calcification rates buoyant weight technique (Jokiel, et al., 1975, Davies 1989).</td>
</tr>
</tbody>
</table>
| **RESPIRATION RATE (mg cm⁻² hr⁻¹)** | Ø Oxygen sensor (YSI multiparameter (Villanueva et al., 2006)  
Ø Surface Area: Paraffin Wax method (Stimzon and Kinzie, 1991) |
| **PHOTOSYNTHETIC EFFICIENCY (Fv Fm⁻¹)** | Diving PAM (Walz, Germany). (Kavousi et al., 2015, Wiedenman et al., 2015) |
| **ZOOXANTHELLAE DENSITY (cell cm⁻² x10⁶)** | Ø Water-pik method (Johannes and Wiebe, 1970)  
Ø Surface Area: Paraffin Wax method (Stimzon and Kinzie, 1991) |
I. SITE SELECTION for ANS EXPERIMENT

Reference Site (R): Malilnep, Bolinao (pH ~8.0)

Intermediate nutrient (IN): Siapar, Bolinao (pH ~7.7)

High nutrient (HN): Salud, Bolinao (pH ~7.6)
II. CORAL COLLECTION and NUBBIN PREPARATION

CORAL COLLECTION: 10 non-gravid adult colonies per species

NUBBIN: 3-4cm (15-18 per colony)

III. ACCLIMATION (for >1 month)

Weekly monitoring (survival) and water sampling (water analyses) at each location. Nubbin recovery for physiological analyses after 56-days

4m² platform in Malinlep

IV. In Situ ANS EXPERIMENT SET-UP

Subsurface (~2-3m) 1m² tray established at each site: (1 per location)

Nubbins deployed for 56-days: 126 per species (14 per tray per location)
Nutrient Stress: RESULTS and DISCUSSION

Response of Adult Coral to ANS
() Survivorship

- All six species have >73% survival at both IN and HN.
- *Pocillopora damicornis* exhibited significantly low survival with 50% at IN and 53% at HN.
Ocean Acidification and Warming: METHODOLOGY

I. Ocean acidification and warming flow-through set-up established in UP MSI-BML Hatchery
II. CORAL COLLECTION and NUBBIN PREPARATION

Coral Collection: 5 non-gravid adult colonies per species

Nubbin: 3-4cm (15-18 per colony)

III. ACCLIMATION (for >1 month)

Treatment replicate: Control

Daily monitoring (system parameters, coral survival) weekly monitor (photosynthetic efficiency: Fv/Fm-1), and weekly water sampling (water analyses).

After 28-day exposure, physiological analyses: 
Growth Rate (% day⁻¹), 
Respiration Rate (mg cm⁻² hr⁻¹), 
Zooxanthellae density (cell cm⁻² x 10⁶)

TREATMENTS

Control: pH 8.1, 28°C

Ocean Warming (T): pH 8.1, 32°C

Ocean Acidification (OA): pH 7.7, 28°C

OAT: pH 7.7, 32°C

Nubbins exposed to treatments for 28 days: 60 per species (5 nubbin per treatment replicate)
Response of Adult Coral to OAT: 2 species
() Survivorship

Survival (Log-rank Test) of both species significantly decrease under thermal stress, and under OAT.
## SUMMARY

### Survival (%)

<table>
<thead>
<tr>
<th>Anthropogenic Nutrient Stress</th>
<th>Low Nutrient</th>
<th>Intermediate Nutrient</th>
<th>High Nutrient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Coral (6 species)</td>
<td>High</td>
<td>Medium to High</td>
<td>Medium to High</td>
</tr>
</tbody>
</table>

### Ocean Warming and Acidification

<table>
<thead>
<tr>
<th>Ocean Warming and Acidification</th>
<th>Ambient</th>
<th>Ocean Acidification</th>
<th>Ocean Warming</th>
<th>OAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Coral (2 species)</td>
<td>High</td>
<td>High</td>
<td>Low to Low-Medium</td>
<td>Low</td>
</tr>
</tbody>
</table>

### Ocean Warming and Acidification

<table>
<thead>
<tr>
<th>Ocean Warming and Acidification</th>
<th>Ambient</th>
<th>Ocean Acidification</th>
<th>Ocean Warming</th>
<th>OAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral Larvae (1 species)</td>
<td>Planulæ stage (10-d exposure)</td>
<td>Medium-High</td>
<td>Medium-High</td>
<td>Medium to Medium</td>
</tr>
<tr>
<td></td>
<td>Planulæ stage (3-d exposure)</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>During metamorphosis (3-d exposure)</td>
<td>High</td>
<td>Medium-High</td>
<td>Medium to Medium-High</td>
</tr>
</tbody>
</table>
In Summary

• Work on OA has started in the Philippines, national program will start

• Current effort to determine baseline information on carbonate parameters utilizes “sites of opportunity”

• On-going work to determine effect of stressors (pH, temperature, nutrients) on corals to come up with list of resilient coral species
  • in support of coral restoration and rehabilitation
Thank you