Standard operating procedures for the deployment, recovery, and analysis of Calcification Accretion Units

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Pacific Islands Fisheries Science Center

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Cover photo: A newly deployed Calcification Accretion Unit. Photo credit: NOAA Fisheries.

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Table of Contents

List of Tables

List of Figures

Introduction

The following document describes the standard operating procedures used to deploy, recover, and analyze calcification accretion units (CAUs) by the Ecosystem Sciences Division at NOAA's Pacific Islands Fisheries Science Center (PIFSC) and the Coral Program at NOAA's Atlantic Oceanographic and Meteorological Laboratory (AOML) as part of the National Coral Reef Monitoring Program (NCRMP; NOAA NCRMP, 2021).

CAUs estimate coral reef calcium carbonate accretion rates by facilitating the recruitment and colonization of crustose coralline algae, hard corals, and other reef calcifiers (Barkley et al., 2022; Vargas-Ángel et al., 2015). Each set of two polyvinyl chloride (PVC) settlement plates is installed on the benthos for up to three years at a time (Figure 1). Following recovery, carbonate accretion rates are estimated by de-calcifying all recruited carbonate material and calculating the change in weight of the CAU plates between deployment and retrieval. Monitoring net accretion over successive deployments allows for the detection of changes in reef calcification rates over time due to ocean acidification and/or other environmental drivers.

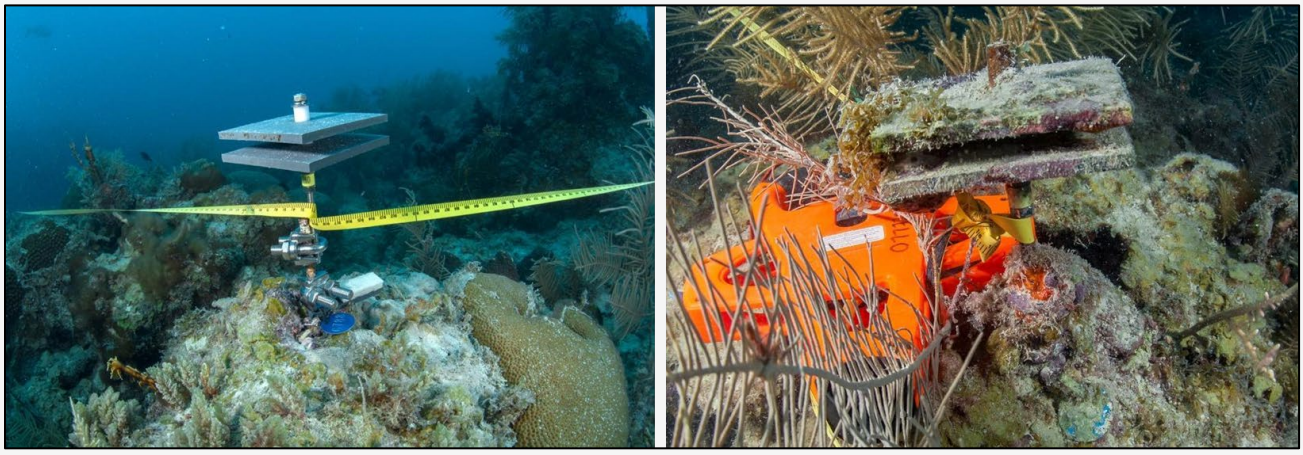

Figure 1. A newly deployed CAU (left) and CAU after a three-year deployment period (right). Bioerosion monitoring units (BMUs) are deployed at the base of the stake.

CAU Assembly

Materials

(2) 4” x 4” x 1/4" grey PVC plates
(1) 1/4-20 x 6” stainless steel full thread rod
(1) seamless pigeon leg band with unique serial number (10mm tall, 8mm ID, 10mm OD)
(3) 1/4-20 stainless steel nuts
(2) 1/4" stainless steel washer
(1) 1/4" stainless steel lock washer
(3) nylon spacer (1/2'' tall, 1/4" ID, 1/2'' OD)
(1) Loctite, or similar thread adhesive product
(1) Aquashield, or similar anti-seize product

Assembly Instructions

Assemble each CAU following the schematic and assembly order in Figure 2.

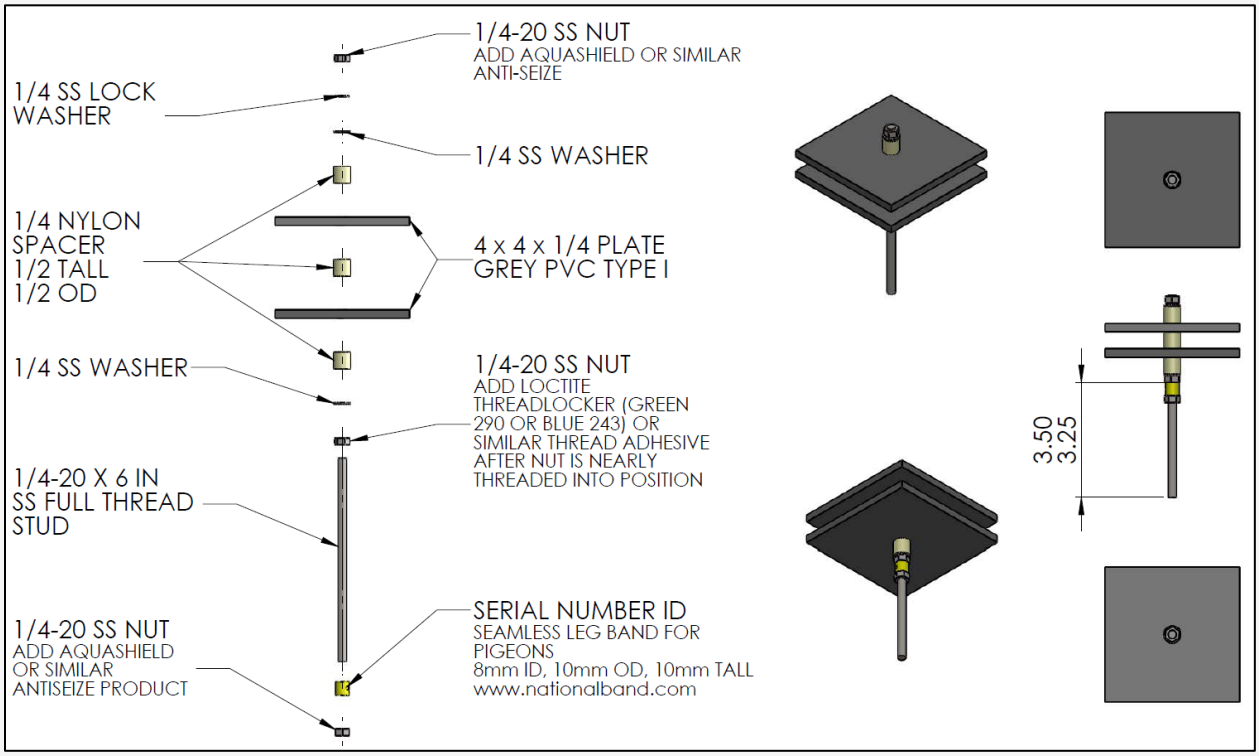

Figure 2. CAU design and assembly instructions. Units are in inches unless otherwise specified.

Field Recovery and Deployment

Materials

(5) assembled CAUs
(5) stainless steel stakes
(1–2) sledge hammers
(5) 11/16” wire rope clips
(1–2) 11/16” socket drivers (recommended) or wrenches
(2) tubes of underwater, 2-part, epoxy
(5) large plastic bags (1 gallon or larger)
(5) 6–8” zip ties, if not using sealable plastic bags
(1–2) mesh dive bags for carrying above listed supplies
(1) 25 lb. lift bag (optional)

Dive Preparation

1. Record the CAU serial numbers to be deployed (found on the yellow pigeon leg bands on an assembled CAU unit; Figure 2) on a field data sheet (Appendix: Example CAU field and laboratory data sheets).
2. Plastic bags can be attached together by securing a zip tie through a small hole punched in the corner of each bag. The five zip ties that will be used to seal the bags can also be “reverse tied” around this center zip tie for storage prior to use.
3. Pack all materials into a mesh dive bag, ensuring that plastic bags are contained and will not float away once underwater.

Dive Operations

1. Locate previously installed CAUs. A weighted marker float dropped on the GPS point can be helpful for finding the deployment site.
2. Recover CAUs by loosening wire rope clips. Wire rope clips will likely be overgrown. Use a socket driver or wrench to clear any fouling (can lightly tap on the clip to dislodge calcified material, taking care to avoid hitting the plates), and then remove the CAU from the stake. Scrape algae off the yellow tag to show the unit serial number.
3. Place recovered CAUs in plastic bags. Seal or secure each plastic bag with a zip tie. Place plastic bags in a mesh dive bag (ideally, separate from the bag containing heavy tools).
4. Attach a new CAU to the upper 3” of the existing stainless-steel stake with a new wire rope clip. Mount with 3” of overlap between the stake and the threaded pin of the CAU.

Use the socket driver to tighten the nuts to hand tight. The CAU should be mounted parallel to the benthos, and the individual CAU plates should be "squared" up (sometimes the two plates can rotate on their mounting pin and not be perfectly positioned on top of each other).

5. If previously installed CAUs are missing or the existing stakes have been compromised / become loose, install a new stake. Use the sledge hammer to drive the stake into a non-living area of reef. Prepare two-part epoxy, and place around the base of the stake where it meets the benthos. Attach CAU to the newly installed stake as described above.
6. Under NCRMP monitoring protocols, CAUs are often co-deployed with bioerosion monitoring units (BMUs). If present, BMUs can be recovered from each stake after CAUs are removed and re-deployed on stakes before new CAUs are installed.
7. Take care when ascending with CAUs to avoid dislodging calcified material. A lift bag can be helpful if the bags of tools and recovered CAUs are heavy.

Post Dive

1. Drain all water from CAU recovery bags and record each recovered CAU serial number and any recovery notes on the field data sheet.
2. Consolidate all recovered CAUs for each site into one large plastic bag. Label bag with site ID and year. Store CAUs in freezer until ready for laboratory analysis.

Laboratory Analysis

The following standard operating procedures document the protocols used to process CAUs at PIFSC. **Bold variables** reference data column names in the CAU data entry spreadsheets (Appendix: Example CAU field and laboratory data sheets). PIFSC uses an Oracle Application Express (APEX) application to record CAU data and calculate calcification rates; however, example data entry spreadsheets and calculations are also provided.

Table 1. Equipment and supplies required for CAU laboratory analysis.

Equipment/Supply	Brand and Model	Specs
Drying oven	Heratherm Advanced Protocol Oven (OGH180, 220-240v, 60Hz) or Heratherm General Protocol Oven (OGS 180, 120v, 60Hz)	Gravity convection; 176 L, 6.2 cu ft; Interior (WxHxD) = 43.69 x 68.07 x 58.83cm (17.2” x 26.8” x 23.2”) Exterior (WxHxD) = 64.01 x 91.95 x 73.91cm (25.2” x 36.2” x 29.1”) Shelves: 2 supplied, 19 max
Digital single-lens reflex (DSLR) camera	Nikon D7000	-
60mm micro lens	Nikon AF-S Micro NIKKOR 60mm f/2.8 G ED	-
Fume hood	-	Benchtop, ducted laboratory hood
Wire mesh shelves	Heratherm Wire Mesh Shelf (Part No.50127763)	Includes 2 shelf support Max carrying capacity/shelf: 24.95kg (55 lbs)
Copy stand	Bencher Copy Mate II Halogen Copystand 900-30SC	Overall dimensions: 50.8cmW x 48.26cmD x 8.89cmH (20"W x 19"D x 3.5"H) Includes two (2) adjustable halogen copylights and two (2) fixed copylight arms: Light output: 300W each, 600W total Bulb: Ushio #1000896 - JCD120V-300WC
Analytical balance	Mettler-Toledo XA Precision Balance (XA503S)	-

Equipment/Supply	Brand and Model	Specs
Vacuum filtration pump	Vacuubrand® Oil-free Diaphragm Pump (ME1) 50/60 Hz	Max pumping speed 0.4/0.5 cfm
Lab-grade rubber tubing	Tygon E-3603 (ACF00029)	Interior diameter = 9.5mm (3/8”) Outer diameter = 15.9mm (5/8”) Wall thickness = 3.2mm (1/8”)
Filter flask	Pyrex	4000 ml
Buchner funnel	Büchner funnel 110 mm, glazed porcelain (Avogadro’s Lab Supply, Inc©, Item # 33065)	Glazed porcelain Inside diameter = 110 mm Height = 172 mm Bowl depth = 44 mm Volume = 456 ml
Rubber filter aid	Rubber filter aid (Avogadro’s Lab Supply, Inc©, Item # FLTR01)	Inside diameter = 32mm (1.2”) Outside diameter = 76mm (3”)
Squeeze bottles	-	-
Tubing connectors	Tapered tubing connector 4.76mm to 7.94mm (3/16” to 5/16”) (Avogadro’s Lab Supply, Inc©, Item # KT511)	Unequal 4.76mm to 7.94mm and 6.35mm to 12.7mm (3/16” to 5/16” and ¼” to ½”)
Büchner funnel mesh discs	Buchner Funnel Discs 90 mm 5/pk (Avogadro’s Lab Supply, Inc©, Item # 242845-90)	Diameter = 90 mm (3.54”) Mesh size = 900 x 700 um for use with 110 mm Büchner funnel
Filter paper	Whatman Qualitative Grade 1 Filterpaper Mfr #1001-110	11 cm diameter Pore size: 11 µm
Razor scraper	Titan 12033	-
Razor blade refills	Garvey (40475)	0.51mm (.02”) thickness 127 x 50.8 x 25.4 mm (5” x 2” x 1”)
Glass dishes	Bormioli Rocco Frigoverre (25 ½ oz) model # 387870MB2321990	15.24 x 15.24 x 6.35 cm (6” x 6” x 2.5”) Capacity = 0.74 L (25.5oz)
Resealable plastic bags	Ziploc® freezer bags - quart size	17.78cm x 19.05cm (7” x 7.75”); Clear; 1.75 ml
Index cards	Pendaflex Oxford Index Cards, White, Blank 12.7 x 20.32cm (5” x 8”)	12.7 x 20.32 x 2.032cm (5” x 8” x 0.8”), white, acid free

Equipment/Supply	Brand and Model	Specs
Hydrochloric acid	MilliporeSigma hydrochloric acid (HCl) (No. HX0603-75); 2.5 L	Molar mass: 36.46; Assay (HCl) approx. 36–38%
Glass bottles for 5% HCl storage	MilliporeSigma storage jugs; 2.5 L	2.5 L; narrow mouth with polypropylene caps
Personal Protective Equipment (PPE)	-	Lab coat Chemical-resistant Disposable gloves Oven mitt Safety glasses Closed-toe shoes
Pencil	-	-
Containers for filter paper	-	-
Tape	-	-
Two wrenches	-	-
Rinse basins for plates	-	-
Mortar and pestle	-	-
Acid-resistant gloves	-	-
Plastic mesh layer (with attached pull string)	-	-
Spatula / teaspoon	-	-
Drying rack for filter paper	-	-
Large plastic bin for HCl waste neutralization	-	-
Arm & Hammer™ baking soda for HCl neutralization	-	-

Prep work

1. Assign a unique ID to both plates of each CAU using the **CAU Serial Plate ID** naming convention:
    a. *SiteID_RecoveryYear_4-digitSerial#_PlateLocation* (*"U" - upper plate, "L" - lower plate; E.g., GUA20_2022_1914_U, GUA20_2022_1914_L*).
    b. Record each **Serial Number** and **CAU Serial Plate ID** in data spreadsheet.
2. Using a pencil, pre-label the following with the **CAU Serial Plate ID***:*
    a. Paper Tray A: blank index card 12.7 x 20.32 cm (5" x 8") folded into 12.7 x 12.7 cm (5" x 5") paper tray
    b. Paper Tray B: blank index card 12.7 x 20.32 cm (5" x 8") folded into 12.7 x 12.7 cm (5" x 5") paper tray
    c. Filter paper: 11 cm diameter, #1, >11 µm particle retention
3. Insert the corresponding filter paper into the fold of "Paper Tray B" and store in a container. Place "Paper Tray A" in a separate container. Store both containers away from moisture and direct sunlight.
4. Print glass dish labels on waterproof paper (example available in in Appendix: Example dish and photo ID labels). Attach each label on one side of the glass dish with tape.
5. Print the plate photo ID tags on waterproof paper (example available in Appendix: Example dish and photo ID labels).
    a. There are 4 tags, one for each surface of the CAU to be photographed:
        i. Upper Plate, Top Side
        ii. Upper Plate, Bottom Side
        iii. Lower Plate, Top Side
        iv. Lower Plate, Bottom Side
    b. An example of a plate photo ID:
        i. *Site: GUA20*
        ii. *Serial N: 1914*
        iii. *Year: 2022*
        iv. *Upper Plate, Top Side*

6. Pre-label re-sealable plastic bags 0.95 L (1 quart) with the **CAU Serial Plate ID**. The filter residue will be stored in this bag for archiving
    a. Using an analytical balance, pre-weigh the following and record the value in grams (up to 3 decimal points):
        i. **Paper Tray Weight** (weight of "Paper Tray A")
        ii. **Filter Paper Tray Dry Weight** (weight of "Paper Tray B" and filter paper)
7. Set the drying oven to the following settings:
    a. Temperature: 60–70°C
    b. Damper: level 3 (mid-high)
8. Prepare 5% HCl acid solution in 2-liter bottles
    a. One batch (20–30 units) of CAUs will require about 10–15, 2 L bottles of 5% HCl acid solution in order to completely dissolve calcified materials. More may be needed depending on the amount of accreted $CaCO_3$.
    b. To dilute full-strength HCl acid (1.18 g/mL, 36.5 molar mass, 37% conc.) into 5% solution, use the recommended ratio below:
    c. 2 L stock: 225 ml HCl: 1775 ml deionized / distilled H_2O
    d. 1 L stock: 112.5 ml HCl: 887.5 ml deionized / distilled H_2O
    e. *Be sure to **ADD ACID TO WATER** to avoid excessive heating, violent boiling or splashing of concentrated acid that could result from the exothermic reaction.
    f. **Acid solutions with a higher (>5% HCl concentration) may be prepared and used if desired for processing CAUs from heavily calcified regions.

Processing Steps

Disassembling and photo documentation

1. Thaw a batch of CAUs (typically 20–30 units) in the sink for at least 12 hours. Do not thaw over water.
2. Disassemble the plates of each CAU by removing the hex nuts and washers using two wrenches (one to hold the unit steady). Make sure to maintain plate identity (upper vs. lower plate, top vs bottom side of plate; Figure 3) and the **CAU Serial Plate ID**.

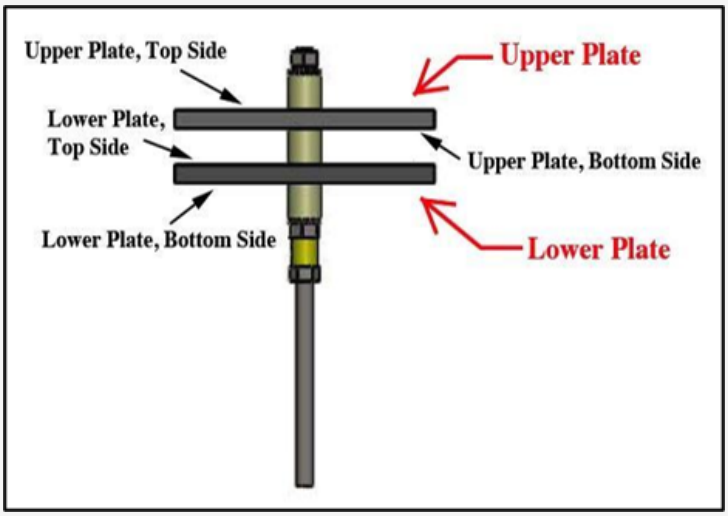

Figure 3. Schematic showing CAU plate identity.

3. Discard any mobile fauna that may have fallen off during rinsing.
4. Place each CAU plate with the top plate facing upwards into its corresponding pre-labeled glass dish (Figure 4).

Figure 4. CAU plates placed in labeled glass dishes.

5. Fill each glass dish with freshwater. Ensure that the plate and any encrusting fauna are completely submerged in water.
6. Attach a DSLR camera to a camera copy stand or a tripod (Figure 5). Arrange lighting such that the plate is fully illuminated and no glare is visible to the camera. Recommend using the Nikon Camera Core Pro V2. Go to “Appendix” under “Camera tethering and CAU plate photo documentation” for detailed steps on using the Nikon Camera Core Pro V2 to photograph CAU plates and auto-save photos in site folders.

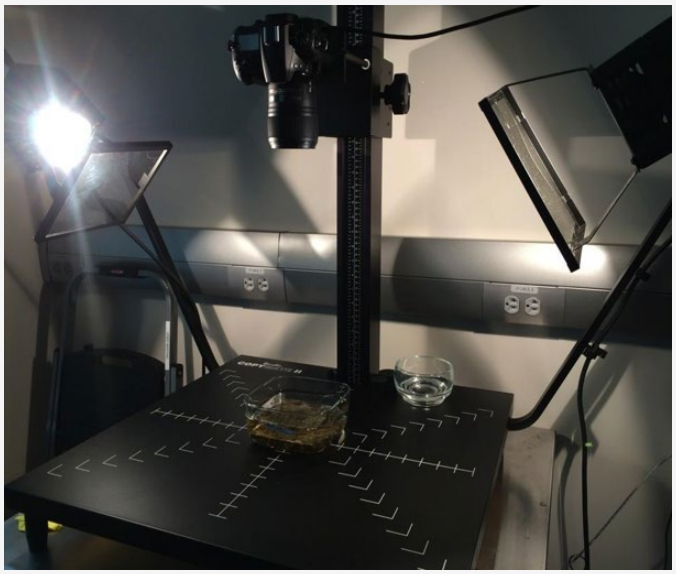

Figure 5. Camera set-up for photographing CAU plates.

7. It is recommended to tether the camera to a computer and save the photos directly to the computer’s local drive (while using the camera SD (secure digital) card as a backup drive). Designate a folder where the CAU photoset will be stored directly.
8. Photograph the top and bottom of each plate, first with the plate photo ID tag and then a second image without the tag (Figure 6). If pieces of calcified material fall off during the photographing process, retain the pieces with the plate to be used during the decalcification process. There should be a total of 8 photos for each CAU.

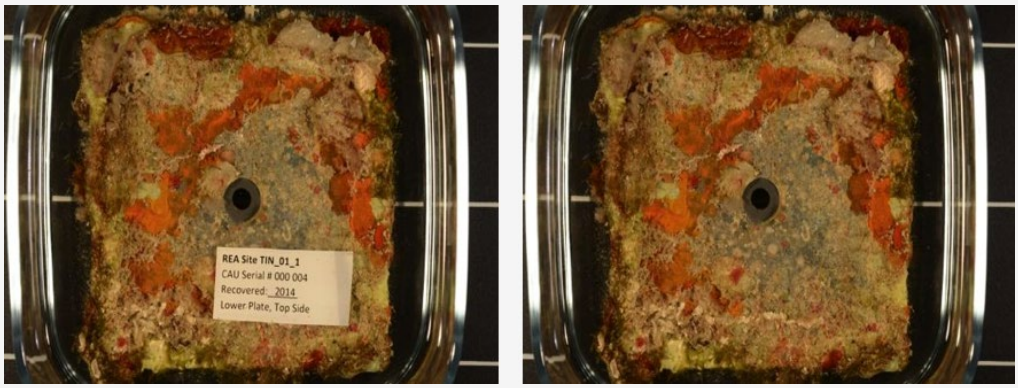

Figure 6. Example CAU photos with (left) and without (right) the plate photo ID tag.

9. After photo documenting, be sure to remove all hex nuts and washers that may be covered by calcified material and difficult to see. Inspect each side of each plate to ensure that there are no washers or hex nuts attached at the center of the plate. If a hex nut or washer is covered in calcified material, remove the hardware and chip off the calcified material from the hardware. Retain the removed carbonate material with the rest of the plate.
10. Place each plate and any dislodged pieces of calcified material on its corresponding "Paper Tray A," then onto a drying rack and placed in the fume hood. The drying rack can be left in a fume hood to air dry at room temperature indefinitely (minimum of 24 hours), until they are dried in the oven. Drying at room temperature before placing in the oven can help reduce the time the plates remain in the oven and slow potential oven rack rusting.
11. Wash and rinse the glass dishes but keep the dish labels on. The same glass dishes will be used during the HCl decalcification process.

Determining the weight of the CAU plate

1. After a minimum air-drying period of 24 hours, place the plate assembly (plate, dislodged pieces of calcified material, and "Paper Tray A") in the oven to dry at 60–70°C for 24 hours. Check that the internal temperature remains consistent. If not, minor adjustments should be made to the oven temperature and damper settings.
2. After 24 hours, remove the plate assembly and wait 10 minutes before weighing.
3. Weigh the entire plate assembly, and record the weight in grams to at least 3 decimal places (**Weight Plate Drying 1**).
4. Return the plate assembly in the oven.

5. Dry the CAU plates for another 24 hours.
6. Remove the plate assembly and set timer for 10 minutes before weighing.
7. Weigh the entire plate assembly, and record the second weight to at least 3 decimal places (**Weight Plate Drying 2**)
8. Compare the two weights:
    a. If the difference in weight is less than 0.1 g, the plates are considered dry and no further drying needs to occur.
    b. If the difference in weight is greater than 0.1 g, return the plates to the oven for another 24 hours.
9. Repeat steps 5–10 until the difference in weight is less than 0.1 g. Add a **column Weight Plate Drying 3+** for each additional weight measured.

Decalcification of calcified material in hydrochloric acid

*Be sure to wear proper PPE when handling acid contaminated objects (steps 1–9) including gloves, safety glasses, and lab coat, and conduct all steps inside of a fume hood.

1. Ensure that the plate is fully dry (mass lost between last two weighing will have been less than 0.1 g) and the final weight of the plate assembly and "Paper Tray A" (**Weight Plate Drying 2**, or last weight measurement) has been recorded.
2. Place the individual plates in the pre-labeled glass dishes. Keep "Paper Tray A" and place under the glass dish.
3. Chip off any large pieces of calcified materials (e.g., crustose coralline algae, coral, shells, etc.) and grind using a mortar and pestle. Return the pulverized forms and plate in the glass dish.
4. Fill the glass dish midway with 5% (or otherwise chosen concentration) HCl solution or until the entire CAU plate is fully submerged in acid. Add HCl slowly as the solution may overflow when bubble formation / decalcification begins.
5. When decalcification has slowed (12–24 hours):
    a. Scrape leftover material from the CAU plates with a razor blade into the glass dish (Figure 7).
    b. Grind pieces of calcified materials that may still be present and return in the glass dish (Figure 7).
    c. Using acid-resistant gloves, sort through the scraped fleshy material in the glass dish and feel for any calcified materials (Figure 7). Some calcified material can be

completely covered by a boundary layer of fleshy material. If present, tease the fleshy material to uncover the calcified material.

d. If there are more calcified materials in the dish, but no obvious decalcification occurring (no bubble formation), decant the used acid solution and drain the fleshy material into another pre-labeled containers (beakers, glass dish, etc.). Add new 5% HCl (or desired concentration) in the first glass dish to continue dissolving the remaining calcified materials.

6. Repeat steps 5b–d until all $CaCO_3$ is fully dissolved. Additional 5% HCl (or desired concentration) may need to be added 2–4 times, depending on the amount of $CaCO_3$ present.
7. Using 5% HCl (or desired concentration) in a squeeze bottle, thoroughly rinse the tools (gloves, mortar and pestle, razor / scraper) into the glass dish.
8. When the CAU PVC plates have been scraped clean (fleshy / calcified material completely removed from either side and edges of the plate), rinse all sides of the plates with 5% HCl (or desired concentration) into the glass dish.

Figure 7. Scraping CAU plates (top), grinding calcified material (middle), and sorting through fleshy material in the glass dishes (bottom).

9. Place the CAU PVC plate on its corresponding “Paper Tray A.”

10. Scrub and rinse the CAU PVC plates in freshwater. Pat to dry. Return to corresponding "Paper Tray A."
11. Place the rinsed CAU PVC plates in the oven at 60–70°C for 24 hours on top of the corresponding "Paper Tray A."
12. Remove the plates from the oven and allow to cool to room temperature for 1 hour (Figure 8).

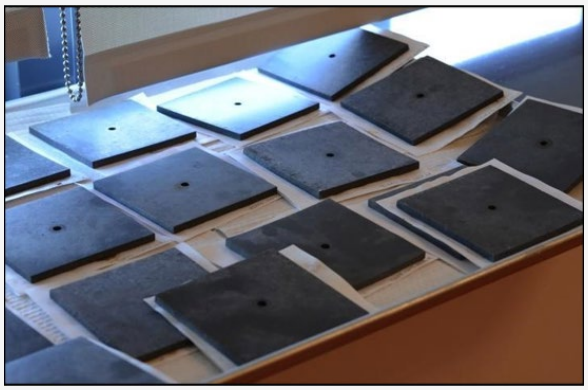

Figure 8. Scraped and rinsed CAU plates cooling on paper trays.

13. Weigh the clean and dry CAU PVC plate without the underlying paper tray, and record the weight in grams to at least 3 decimal places (**CAU PVC Dry Weight**). "Paper Tray A" can be discarded after the CAU PVC plates have been weighed and recorded.
14. Collect the clean and weighed CAU PVCs and store for redeployment.

Vacuum filtration of fleshy material

*Be sure to wear proper PPE when handling acid-contaminated objects (steps 1–4) including gloves, safety glasses, and lab coat.

1. Using a 4-liter Büchner funnel, a plastic mesh layer (with attached pull string) and the corresponding pre-weighed filter paper, vacuum filter the acid bath and remaining fleshy material for each CAU plate (Figure 9). Use a freshwater squeeze bottle to rinse contents of the glass dish into the funnel.

Figure 9. Vacuum filtration of fleshy material using Buchner funnel.

2. If the volume of fleshy material is sufficient to clog the filter paper, a second pre-labeled and pre-weighed filter paper can be used to filter the remaining volume of acid bath and fleshy material:
    a. Label an additional filter paper and paper tray with its corresponding **CAU Serial Plate ID**.
    b. Record the weight of the second filter paper and paper tray in grams to at least 3 decimal places (**Additional Filter Paper Dry Weight**).
3. If filtering has slowed down, use a spatula / teaspoon to gently scrape the surface of the filter paper to allow more solution to filter through. Filtering can take >5 mins depending on the amount of fleshy material in the acid bath.
4. When filtration is completed, place the filter paper on its corresponding “Paper Tray B” and onto a drying rack (Figure 10). The drying rack should be left in a fume hood to air dry at room temperature (minimum of 24 hours). The filter paper can continue to air dry at room temperature, indefinitely, until they are dried in the oven.

Figure 10. Filter paper with fleshy material drying on paper tray.

Determining the weight of the fleshy material

*Be sure to wear proper PPE when handling acid-contaminated filters in this section.

1. After a minimum air-drying period of 24 hours, place the filter paper assembly (filter paper and "Paper Tray B") in the oven to dry at 60–70°C for 24 hours. Check that the internal temperature remains consistent. If not, minor adjustments should be made to the oven temperature and damper settings.
2. After 24 hours, remove the filter paper assembly and wait 10 minutes before weighing.
3. Weigh the entire filter paper assembly (Figure 11), and record the weight in grams to at least 3 decimal places (**Filter Paper Drying 1**).
4. Return the filter paper assembly to the oven.
5. Dry the filter paper for another 24 hours.
6. Remove the filter paper assembly and wait 10 minutes before weighing.
7. Weigh the entire filter paper assembly.
8. Record the weight in grams to at least 3 decimal places (**Filter Paper Drying 2**).
9. Compare the current weight from the previous weight:
    a. If the difference in weight is less than 0.1 g, the plates are considered dry and no further drying/weighing needs to occur.
    b. If the difference in weight is greater than 0.1 g, return the plates to the oven for another 24 hours.
10. Repeat steps 5–10 until the difference in weight is less than 0.1 g.

Figure 11. Weighing filter paper.

11. After recording the final filter paper assembly dry weight, place the filter paper and "Paper Tray B" in its corresponding re-sealable bag for archiving (Figure 12). Ensure the bag is labeled properly with the **CAU Serial Plate ID**.

Figure 12. Filter papers stored in labeled plastic bags for archiving.

Data Processing

The following data are calculated for each CAU plate:

- **Final Material Dry Weight** (g) **=**

  Weight Plate Drying 2 (or the final plate weight collected, g) –
  Paper Tray Weight (g) –
  CAU PVC Dry Weight (g)

- **Final Fleshy Weight** (g) **=**

  Filter Paper Drying 2 (or the final filter weight collected, g) –
  Filter Paper Tray Dry Weight (g) –
  Additional Filter Paper Dry Weight (if used, g)

- **Net Weight** (g) **=**

  Final Material Dry Weight (g) –
  Final Fleshy Weight(g)

- **Years in Water =** number of decimal years between CAU deployment date and CAU recovery date

- **Cal Rate by Plate** (g cm^{-2} yr^{-1}) **=**

  Net Weight (g) /
  200 (surface area of plate, cm^2) /
  Years in Water

Cal Rate by Plate is then averaged across the two plates for each CAU **Serial Number** to calculate **Cal Rate by Unit.**

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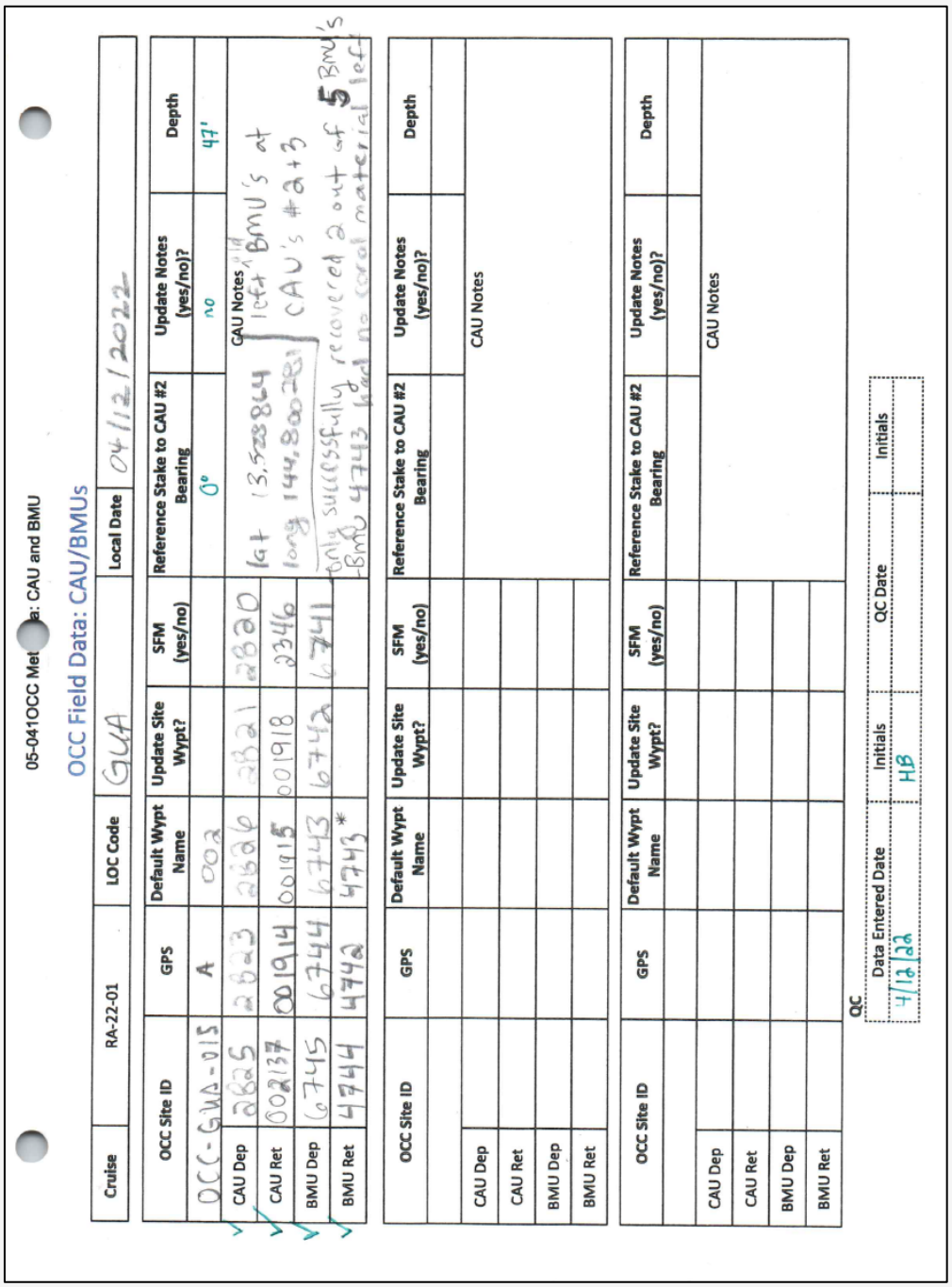
Appendix

Example CAU field and laboratory data sheets



05-041OCC Met ...a: CAU and BMU

OCC Field Data: CAU/BMUs

Cruise	RA-22-01	LOC Code	GUA	Local Date	04/12/2022

OCC Site ID	GPS	Default Wypt Name	Update Site Wypt?	SFM (yes/no)	Reference Stake to CAU #2 Bearing	Update Notes (yes/no)?	Depth
OCC-GUA-015	A	002			0°	no	47'
CAU Dep	2625	2606	2821	28820	lat 13.523864 long 144.800281	GAU Notes Left BMU's at CAU's #2+3 only successfully recovered 2 out of 5 BMU's BMU 4743 had no coral material left	
CAU Ret	001974	001915	001918	23446			
BMU Dep	6745	6744	6743	6742			
BMU Ret	4744	4742	4743*				

OCC Site ID	GPS	Default Wypt Name	Update Site Wypt?	SFM (yes/no)	Reference Stake to CAU #2 Bearing	Update Notes (yes/no)?	Depth
CAU Dep						CAU Notes	
CAU Ret							
BMU Dep							
BMU Ret							

OCC Site ID	GPS	Default Wypt Name	Update Site Wypt?	SFM (yes/no)	Reference Stake to CAU #2 Bearing	Update Notes (yes/no)?	Depth
CAU Dep						CAU Notes	
CAU Ret							
BMU Dep							
BMU Ret							

QC

Data Entered Date	Initials	QC Date	Initials
4/13/22	HB		

Figure 13. Example field data sheet used by PIFSC (including both CAUs and BMUs) for CAU deployments and recoveries.

Table 2. Example data entry table used for CAU laboratory analysis. All weights are in grams.

Serial Number	CAU Serial Plate ID	Paper Tray Weight	Filter Paper Tray Dry Weight	Additional Filter Paper Dry Weight	CAU PVC Dry Weight	Weight Plate Drying 1	Weight Plate Drying 2	Filter Paper Drying 1	Filter Paper Drying 2
1914	GUA-20_1914_U	3.121	4.011	-	92.597	109.321	109.205	4.710	4.690
1914	GUA-20_1914_L	3.173	4.003	-	93.145	127.929	127.798	7.266	7.086
1915	GUA-20_1915_U	3.154	4.001	-	90.972	115.255	115.103	6.349	6.293
1915	GUA-20_1915_L	3.124	4.021	-	94.299	122.625	122.545	5.258	5.199
1918	GUA-20_1918_U	3.106	4.009	-	92.651	109.218	109.132	5.354	5.292
1918	GUA-20_1918_L	3.919	3.980	-	92.546	117.732	117.642	5.300	5.232
2137	GUA-20_2137_U	3.122	3.971	-	93.836	106.538	106.475	4.479	4.453
2137	GUA-20_2137_L	3.152	3.998	-	91.771	203.522	202.334	7.130	7.105
2346	GUA-20_2346_U	3.133	3.961	-	91.601	110.397	110.306	5.645	5.535
2346	GUA-20_2346_L	3.125	3.998	-	93.732	120.465	120.339	5.536	5.475

Table 3. Example of data table with calcification rate per plate and per unit calculations.

Serial Number	CAU Serial Plate ID	Final Material Dry Weight (g)	Final Fleshy Weight (g)	Net Weight (g)	Years in Water	Cal Rate by Plate (g cm^{-2} yr^{-1})	Cal Rate by Unit (g cm^{-2} yr^{-1})
1914	GUA-20_1914_L	31.480	3.154	28.326	4.94	0.029	0.021
	GUA-20_1914_U	13.487	0.679	12.808	4.94	0.013	
1915	GUA-20_1915_L	25.122	1.178	23.944	4.94	0.024	0.022
	GUA-20_1915_U	20.977	2.292	18.685	4.94	0.019	
1918	GUA-20_1918_L	21.177	1.252	19.925	4.94	0.020	0.016
	GUA-20_1918_U	13.375	1.283	12.092	4.94	0.012	
2137	GUA-20_2137_L	103.372	3.107	100.265	4.94	0.101	0.055
	GUA-20_2137_U	9.517	0.482	9.035	4.94	0.009	
2346	GUA-20_2346_L	23.482	1.477	22.005	4.94	0.022	0.018
	GUA-20_2346_U	15.572	1.564	14.008	4.94	0.014	

Camera tethering and CAU plate photo documentation

This section is for the use of Nikon Camera Control Pro 2. Refer to the brand / model user manual if using a different camera and setup.

1. Install the software for the Nikon Camera Control Pro 2.
2. Connect Nikon DSLR camera (Nikon D7000 with AF-S Micro NIKKOR 60mm f\2.8G ED lens) to the computer via USB mini cable.
3. Start the Nikon Camera Control Pro 2 application and turn the camera ON.
4. Once connected, the connection status of the camera in use will be displayed on the Camera Control Pro window.
5. If using the software for the first time, under the "Camera Control" tabs:
    a. Click on "Exposure 1" and change these settings:
        i. Shutter speed: 1/50 sec
        Aperture: f/8
    b. Click on "Exposure 2" and change these settings:
        i. ISO Sensitivity: ISO 400
        ii. White Balance: Auto
        Option: Normal
    c. Click on "Storage" and change these settings:
        i. Image quality: JPEG fine
        JPEG compression: Optimal quality
        Image size: Large (4928x3264)
        Record to: PC
        Card record mode: Backup
    d. Click on "Image Processing" and change these settings:
        i. Picture control: Standard
        High ISO NR: On (High)
6. Ensure the camera's Mode dial is set to "M" (manual).
7. Ensure the camera lens' A-M is set to "M/A" (autofocus with manual override).
8. Go to the "Tools" menu to display the "Transfer Options" window.
9. Click the "Browse" button next to the "Destination folder." Navigate to the appropriate folder to where the CAU photos will be saved. Press "OK."

10. Click the “Edit” button next to the “File to be used”. Rename the file name with the following naming convention: *“SiteID_RecoveryYear_4-digitSerial#_”* Set to three digits, and start at 1. This will save your photos in sequential order starting at 001 and ending at 008. There should be a total of 8 photos taken for each CAU, e.g.:

    GUA20_2022_1914_001.jpg
    GUA20_2022_1914_002.jpg
    GUA20_2022_1914_003.jpg
    GUA20_2022_1914_004.jpg
    GUA20_2022_1914_005.jpg
    GUA20_2022_1914_006.jpg
    GUA20_2022_1914_007.jpg
    GUA20_2022_1914_008.jpg

    **Note: Step 10 will need to be repeated for each CAU photographed.*

11. On the “Camera Control Pro” main window, click on the “LV” button at the bottom-right corner to display the Live View window.
12. Center the glass dish with the plate under the camera and using the “Live View” display, ensure that all corners of the plate are visible in the frame.
13. Turn on stand lights and minimize any external light sources. Using the “AF and Shoot” button on the “Live View” window, photograph the top of the plate, first with the plate photo ID tag, second image without the tag. Repeat this step for the bottom plate.
14. When finished with one full CAU (8 images), exit out of the “Live View” window and rename the next CAU following Step 10.
15. At the end of the plate photo documentation session, ensure that all CAU plate photos have been taken and saved to their appropriate local drive folders.

Example dish and photo ID labels

Table 4. Example ID labels for CAU dishes and plates.

Site GUA_20	**Site GUA_20**	**Site GUA_20**	**Site GUA_20**
CAU Serial # 1914	CAU Serial # 1914	CAU Serial # 2137	CAU Serial # 2137
Recovered: 2022	Recovered: 2022	Recovered: 2022	Recovered: 2022
Upper Plate, Top Side	Upper Plate, Bottom Side	Upper Plate, Top Side	Upper Plate, Bottom Side
Site GUA_20	**Site GUA_20**	**Site GUA_20**	**Site GUA_20**
CAU Serial # 1914	CAU Serial # 1914	CAU Serial # 2137	CAU Serial # 2137
Recovered: 2022	Recovered: 2022	Recovered: 2022	Recovered: 2022
Lower Plate, Top Side	Lower Plate, Bottom Side	Lower Plate, Top Side	Lower Plate, Bottom Side
Site GUA_20	**Site GUA_20**	**Site GUA_20**	**Site GUA_20**
CAU Serial # 1915	CAU Serial # 1915	CAU Serial # 2346	CAU Serial # 2346
Recovered: 2022	Recovered: 2022	Recovered: 2022	Recovered: 2022
Upper Plate, Top Side	Upper Plate, Bottom Side	Upper Plate, Top Side	Upper Plate, Bottom Side
Site GUA_20	**Site GUA_20**	**Site GUA_20**	**Site GUA_20**
CAU Serial # 1915	CAU Serial # 1915	CAU Serial # 2346	CAU Serial # 2346
Recovered: 2022	Recovered: 2022	Recovered: 2022	Recovered: 2022
Lower Plate, Top Side	Lower Plate, Bottom Side	Lower Plate, Top Side	Lower Plate, Bottom Side
Site GUA_20	**Site GUA_20**	**Site GUA_20**	**Site GUA_20**
CAU Serial # 1918	CAU Serial # 1918	CAU Serial # 1918	CAU Serial # 1918
Recovered: 2022	Recovered: 2022	Recovered: 2022	Recovered: 2022
Upper Plate, Top Side	Upper Plate, Bottom Side	Lower Plate, Top Side	Lower Plate, Bottom Side