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.

ARMS units in aerated disassembly tubs.

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.

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.

ARMS Processing
- Transfer and Disassembly
- Photography
- Brushing
- Sieving
- Sorting and Documentation
- Scraping
- Processing Products
Detaching the latching straps (left) and flushing the mesh liner with sea water to detach organisms (right). 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.

Removing the jam nuts connecting the base plate to the tier of plates (left) and jam nuts that hold the tier of ARMS plates together (right).

**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.

Photographing the whole plate (left), taking close-up photos (upper middle), an image of labeled plate (lower middle), and an image to be used for analysis (right).
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.

Photographing the brachyuran crab, *Lybia edmonsoni*.

**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.

Scraping the sessile materials off of the plates.
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.