ARMS PROTOCOL

Note – on Day 1 collect just one ARMS to get bugs out of system. Generally two ARMS per day is a maximum for full protocol

Step 1: FIELD COLLECTION

Remove the stakes and put a small cable tie in the northern corner of the baseplate (Figure 1). Place lined crate (mesh size 100 μm) (Figure 2) over ARMS and fasten with hooked elastic cords. Swim up to boat and place in container with seawater and cover. Remove crate (Figure 3)

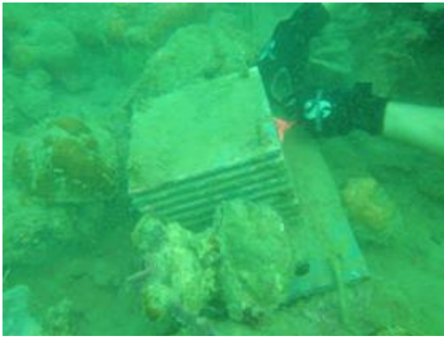

Figure 1. Northern corner on baseplate

Figure 2. Lined crate to cover the ARMS and fasten with hooked elastic cord.

Figure 3. ARMS stored in action packer for carrying them in the boat, with sea water and aeration. Cover the container when possible.

Step 2: DISASSEMBLY

Remove the crate from the ARMS (as soon as possible) and put in at least two aeration stones. Wearing long gloves turn the ARMS on side and unscrew each of the nuts and bolts that are holding the ARMS in place at each corner (Figure 4) , but do not remove the long bolt (remove all of them at the same time). Put the ARMS back in the crate and remove baseplate. Put a small cable tie in the northern corner of each plate (this will be taken into account for microbes).

Rinse and examine the nitex-lined crate (the one used to cover the ARMS underwater) for any hiding organisms (and in case of some organisms is there, put it back in the action packer, where the ARMS is).

Figure 4. Disassemble the ARMS and brush the plates separately, as they are disassembled.

Brush baseplate minimally and leave it a part. One by one lightly brush each plate (both sides) and then put in 10 L bucket (only 1 plate per bucket), use latex gloves to carry. Buckets should have at least one air stone. Put a label in bucket for each plate surface to identify top and bottom sides (if top and bottom sides are well differenced - top less organisms due to the sediment deposition and bottom highly cover by organisms – no label is necessary). Label the bucket with plate number.

Step 3: PHOTODOCUMENTATION OF PLATES

By the time each plate is disassembled, put it in a trey filled with seawater. Each plate surface is photographed overall and in close-up of each quadrant and the center (Figure 5). Photograph the overall shot with a label (Put the label ARMS # and plate #_ top/bottom and take the first picture with it, so the following pictures belong to it, until next label).

Figure 5. Plate in the tray to be photographed.

Step 4: PROCESSING MOBILE FRACTIONS

Take the coarse (1000 µm) and medium (500 µm) sieve and pour all the liquid (the water that is still in the action packer) from the crate through the two sieves (Figure 6). To get last bits of sediment out, wash with the water that has passed through the sieves. Place contents of these two sieves in shallow trays (labeled previously with the size of the sieve) - if fragile large organisms come out early transfer to >2mm mobile tray to avoid damaging them. Put aeration in the trays. Then, take the medium (500 µm) and fine (100 µm) sieve and repeat the process with the water that passed through the coarser two sieves. If there is too much very fine sediment to process, then make sure to mix muddy water very well so that it is suspended homogeneously before discarding remaining sediment. Tap the bottom of sieve to facilitate drainage and sometimes it may be necessary to put the contents of the sieves in the trays and then continue if the amount of sedimentary material is a lot. You do not need to keep debris such as oyster shells and seagrass clumps that have clearly fallen off the baseplate. Use gloves throughout.

Figure 6. Sieves for filtering.

Step 5: PHOTOS AND PRESERVING THE MOBILE FRACTIONS

>2mm: If there is time sort completely and photograph individually (label with ARMS number). If not, bulk photo and bulk preserve in 95% ethanol, label, and put in freezer (Figure 7).

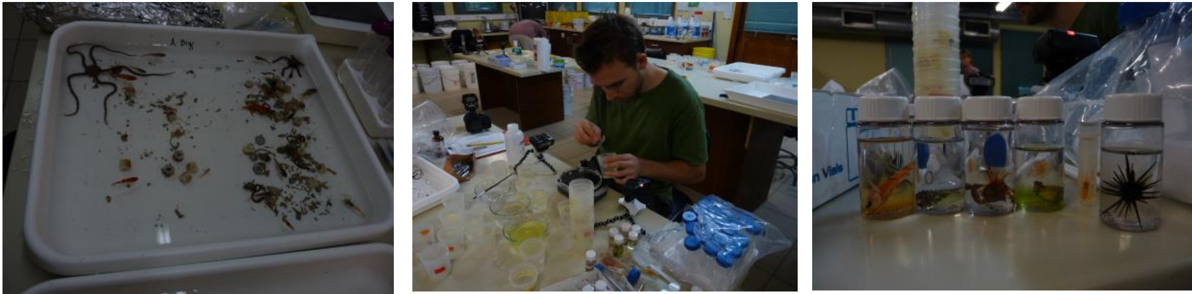

Figure 7. Left: big fraction after sieving, disposed in tray with sea water. Middle: sort and photograph organisms. Right: preserve organisms individually in ethanol (labeled).

2mm-500 um: Pour the sample through 2-piece plastic tube with a fresh piece of nitex (40 µm) in between the two pieces (or alternatively into nitex net (40 µm) – such as the net used for bulk scraped fraction) (Figure 8). Rinse with ethanol 95% to remove more seawater. With the help of a spatula put material from nitex into 50 ml falcon tubes (depending on the amount use several tubes) and add 95% ethanol (proportion: 20% sample – 80% ethanol). Label, put tubes in a plastic bag (label) and freeze.

Rinse in bleach the material before processing next fraction.

500-100 um: pour into nitex net, squeeze out water, and rinse with 95% ethanol. Place in 50 ml falcon tubes and add 95% ethanol, label, put tubes in a plastic bag (label) and freeze.

Figure 8. Rinse the sample with ethanol through 2-piece plastic tube with a piece of nitex.

Step 6: SUBSAMPLING THE SESSILE FRACTION

MICROBES
Take the following sides of the plates:

1 top
2 bottom + 3 top (open)
3 bottom + 4 top (closed)
4 bottom + 5 top (open)
5 bottom + 6 top (closed)
6 bottom + 7 top (open)
7 bottom + 8 top (closed)

Randomly select three squares to be subsampled. Place the plate in a numbered trey and from each selected square subsample three samples as it is detailed in Figure 9.

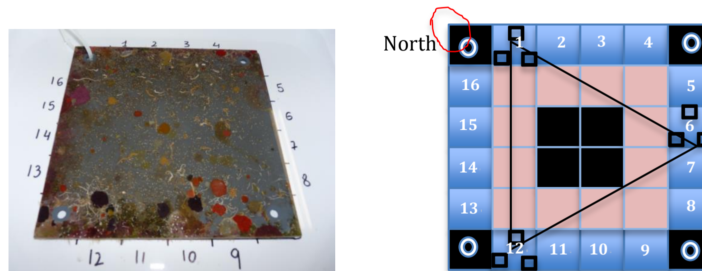

Figure 9. Place the plate in a tray with seawater for microbes sampling (left). Microbes sampling scheme for the different plates (right).

Using a scalpel scrap each square and place the material sampled in a 5 ml vial previously filled with RNA later and labeled (ARMS#_plate#_top/bottom_square#a/b/c) (Figure 10). Even if the sub-square does not have any visible material it has to be sampled. Scalpel needs to be rinsed in alcohol and flamed between each sample. Fill vials with 1 ml of RNA later. **Prep Work before leaving**: label and fill tubes.

Figure 10. Microbes sampling.

SESSILE ORGANISMS

After microbes sampling, use a random number or gridded sampling to subsample specific locations on the plate. These can be photographed if individually numbered at end of subsampling. In addition or alternatively, photo and voucher small areas of distinctive sessile organisms with numbers. Preserve samples in 95% ethanol. Scalpel needs to be rinsed in alcohol and flamed between each sample.

Step 7: PRESERVING THE BULK SCRAPED FRACTION

Note: if ethanol in adequate amounts is available, use this rather than seawater.

Scrape the surfaces and sides of all pvc plates with a scraper into a tray of seawater or ethanol (but not the baseplate). Wash the scraped fraction into a nitex net and squeeze out with seawater or ethanol (Figure 11).

Figure 11. Left and middle: after scraping, wash the fraction into a nitex mesh. Right: squeeze out with ethanol.

Place material in blender with seawater or ethanol as needed (roughly 1:1 in volume) and blend for ~5 seconds depending on quantity and coarseness. Pour the blend back into a nitex mesh and wash thoroughly (at least 5 times, sometimes much more depending on the mucous in the material) with fresh seawater or ethanol, squeezing out the liquid through the mesh each time (Figure 12). If using seawater, it will start out foamy but when clean will be noticeably less foamy (modest number of bubbles at the surface).

Figure 12. Blend of the scraped fraction. During 5 seconds with a proportion 1:1 sample:ethanol. After blending, pour the blend back into a nitex net and rinse with ethanol (several times).

After last wash and thorough squeezing out of liquid, place a small scoop (~teaspoon) in 50 ml falcon tube and then fill with DMSO (25% DMSO) or 95% EToH + EDTA. Fill at least 3 tubes, more if material is substantial (Figure 13). Remainder can be frozen in whirlpacks if more than 4 tubes are needed.

Figure 13. After squeezing out the liquid, place the sample into 50 ml falcon tube up to 10 ml, fill with DMSO and shake vigorously.

MATERIAL FOR ARMS

Step 1: FIELD COLLECTION

- Lined crate for recovery (mesh size 100 µm)
- Three elastic cords per ARMS
- Cable tie
- Container (action packer) for carrying ARMS in boat
- Buckets for filling containers and bringing clean seawater to lab
- Nitex filter for filtering seawater
- Sea ice (if necessary)

Step 2: DISASSEMBLY

- Aerators and aeration stones (13: 10 plates plus 3 mobile fractions
- Wrenches
- Paintbrush
- Long gloves
- Latex gloves
- Labels (ARMS # and plate #_ top/bottom for each plate
- 10 buckets

Step 3: PHOTODOCUMENTATION OF PLATES

- Camera and 2 strobes (luces)
- Tripod
- Labels

Step 4: PROCESSING MOBILE FRACTIONS

- Gloves
- 3 sieves (100, 500 and 1000 µm)
- 3 trays (plus 1 in case is needed for >2mm organisms)
- Aerators and stones
- Second large container for the water
- Squirt bottle for seawater to rinse material off sieves, etc.
- Plastic bags

Step 5: PHOTOS AND PRESERVING THE MOBILE FRACTIONS

- Nitex net (40 µm)
- Falcon tubes (50 ml): 8 total per ARMS
- Bleach (10%)

- Bucket for bleach
- Labels (ARMS number and fraction size)
- Ethanol 96% in squeeze bottle
- Squeeze bottle
- Latex gloves
- Waterproof paper for labels
- Spatula
- Plastic bags

Step 6: SUBSAMPLING THE SESSILE FRACTION

- Latex gloves
- 5 ml vials (117 per ARMS for microbes)
- 5 ml vials (for sessile organisms)
- RNA later
- Scalpels
- Alcohol lamp / alcohol flame (one for each 2 people)
- Plastic bags
- Ethanol 96%
- Glass flask
- Box (to store the tubes)
- Camera
- Labels
- Plastic bag

Step 7: PRESERVING THE BULK SCRAPED FRACTION

- Scrapers (Painters tool)
- Nitex net (40 µm)
- Gloves
- Squeeze bottle
- Blender
- Falcon tubes (50 ml). 4 total per ARMS
- Whirlpaks
- Bleach
- Bucket for bleach
- Labels
- DMSO or EtOH+EDTA

ADDITIONAL INFO

Decontamination - between uses, blender, sieves and nets need to be rinsed in 10% bleach solution (mixed with fresh water) and then VERY thoroughly rinsed in fresh water. Do not leave nets in bleach for more than 30 sec.

MOVING AND HOLDING SAMPLES

10 liters buckets – 10 per ARMS for simultaneous processing plus 10 for seawater/EtOH rinses/bleach solution etc.: **20 total**

Aerators and airstones/tubing: enough for 13 plates/fractions per ARMS being simultaneously worked on: **15-20 connections and at least 2 aerators**

Plate trays – 3 for each of the motile fractions, 1 for photography, 1 for ethanol rinse, 1 per person for subsampling (marked with grids if no frame available): **10 total**

SAMPLE PROCESSING

3 sieves

4 nitex nets (40 µm) (3 per ARMS-mobile 1, mobile 2, scrapings, plus an extra)

Blender

Squirt bottles: 2 for filtered seawater, 2 for ethanol, 1 for misc. (get good brand):**5 total**

PRESERVING

RNA later: 300 ml per ARMS (1 ml per subsampling tubes-117ml)

DMSO (or ETOH + EDTA): 200 ml per ARMS (4 falcon for scrapings at 50 ml each)

Ethanol: calculate necessary volume

5 ml vials: 117 per ARMS

50 ml falcon tubes: 12 per ARMS processed

Whirlpacks: 4 per ARMS

PHOTOGRAPHY

Camera, tripod, and two flashes – 2 sets

Small cups – 50

Computer

MISCELLANEOUS

Kimwipes: 4 boxes

Marking tape: 2 rolls (narrow and wide)

Cable ties: 25 total per ARMS (20 small, multi-color)

Latex gloves: 10 pairs per person per day (medium and large mostly)

Wrenches – 2 sets

Permanent marker: 1 per person

Pencil for labelling