



Handbook

for

Marine Biodiversity Observation network for genetic monitoring of hard-bottom communities (ARMS-MBON),
Operating as part of EMO BON

Version
3

Date
2024 - 04 - 18

Table of Contents

1. Summary	3
2. Joining ARMS-MBON/EMO BON	4
3. Setting up your observatory	4
3.1. Choosing observatory sites and deployment periods	4
3.2. Purchasing ARMS	5
4. Identifiers used in ARMS-MBON	5
4.1. Sample terminology	6
4.2. Replicates	7
4.3. Sampling sites in one observatory	8
5. Deployment and retrieval	8
5.1. Assembly	8
5.2. Deployment	9
5.3. Retrieval	9
6. ARMS processing	10
6.1. Disassembly	11
6.2. Photographing	12
6.3. Manual observations	13
6.4. Vouchering	13
6.5. Collection of fractions (Material Samples)	13
6.5.a. Motile fractions (MF100 and MF500)	13
6.5.b. Sessile fractions (SF40)	15
6.6. Blank samples	16
6.7. Preservation	16
7. Digital processing of ARMS data	16
7.1. Image data	16
7.2. Manual observations	17
8. Shipment	18
8.1. Shipping times and address	18
8.2. Checklist for the sample package	19
8.3. What happens next?	19
9. Biobanking	19
10. Data management	20
11. Contacts	20
12. Links	20
13. Checklist	21

1. Summary

This handbook is an extension of the ARMS-MBON handbook that was in operation during the ASSEMBLE Plus project years of ARMS-MBON (2018 to 2022). As of summer 2022, the ARMS-MBON efforts became part of the European Marine Omics Biodiversity Observation Network ([EMO BON](#)), an [EMBRC](#) initiative, and this has necessitated a few changes. The IDs for events and samples, and the shipping and sequencing steps, have all changed. Also, during the five first years of ARMS-MBON, the network collectively gained experience with ARMS and methods have been progressively adapted for better data gathering and processing. This is all detailed in this version of the handbook.

This handbook provides a compilation of the standards required for setting up observatories as part of the Marine Biodiversity Observation Network for genetic monitoring of hard-bottom communities (ARMS-MBON) operating within EMO BON. This document collects the guidelines, protocols, and recommendations for: the deployment and retrieval of ARMS observatories as established and further developed by the network; AMRS processing and sample preparation, including shipment and biobanking of samples; structuring the collected information (genetic data, image data, *in situ* measurements and recordings of biological and environmental variables) and the metadata and associated legal documents.

The EMO BON Handbook and other documentation can be found in the EMO BON [website](#). All EMO BON documentation is additionally openly accessible through the Ocean Best Practices System repository (<https://repository.oceanbestpractices.org/handle/11329/1735>).

ARMS are highly standardised passive collectors for the assessment of epibenthic and hyperbenthic marine communities. ARMS units are monitoring systems originally developed during the Census of Marine Life project for the collection of marine fauna on and near the sea floor. They are stacks of settlement plates that are fixed to each other and to a stable base. They are deployed on marine substrates and colonised by marine species. After a period of time, they are recovered and taken apart to see which species colonised them. In ARMS-MBON, the units are deployed at different locations, and for various periods, depending on the scientific question being addressed and the types of ecosystems studied (*e.g.* polar regions usually need longer deployments).

The ARMS project began in 2018 under [ASSEMBLE Plus](#) as part of its Genomics Observatories Joint Research Activity. The ARMS project became part of GEO BONs Marine Biodiversity Observation Network ([MBON](#)), and it then adopted the name ARMS-MBON. ASSEMBLE Plus ended in 2022, but ARMS-MBON was then absorbed into the [EMO BON](#) project, run by [EMBRC](#).

Changes since the last release:

1. Comprehensive integration of all steps from deployment to sample shipping within this handbook, making it the go-to guide for ARMS-MBON protocol procedures, excluding Smithsonian protocols.
Introduction of detailed protocols covering assembly, deployment, retrieval, disassembly, photography, manual observation, fractions collection, blank samples, sample preparation, and preservation.
Note: These updates may introduce variations from previously followed methods; users are encouraged to review the new protocols carefully.
2. Three “field replicates” for each sampling site are highly recommended. All references to “independent replicates” have been removed.
3. ARMS-MBON deployments are not categorised in either NIS monitoring OR long-term biodiversity monitoring. Minimum deployment duration is set at 3 months for temperate areas but does not depend on this previous categorisation anymore.

2. Joining ARMS-MBON/EMO BON

The partners we can accept into EMO BON (and hence into ARMS-MBON) are limited because of the funding. However, while you may not be able to formally join either network, we do welcome “expressions of interest”: either for working together on aspects of genomics observatories, or if you want to set up an ARMS network in your locality/region/country/continent. An expression-of-interest form is under development (it will be somewhere on the [EMO BON website](#)), but meanwhile you can send an email to: Matthias Obst (ARMS-MBON coordinator) matthias.obst@marine.gu.se, or the EMO BON secretariat: emobon@embrc.eu

3. Setting up your observatory

Before going ahead with ordering your ARMS unit(s), a certain amount of preparation is necessary.

3.1. Choosing observatory sites and deployment periods

Finding the area of interest. We encourage our partners to establish “observatory sites”, which are localities where ARMS can be re-deployed on a regular basis, and hence build up a time series. Choosing a site that is accessible will allow for regular deployment and retrieval and can support non-indigenous species detection or Long-term ecological research. Examples are marinas, ports, Marine Protected Areas (MPAs), LTER sites, or areas with high oceanographic connectivity. You should expect to deploy and retrieve ARMS at regular intervals depending upon the needs of your observatory.

Finding the right spot in the area of interest. Finding a good spot for long-term monitoring is not easy. Sometimes there are places where regular biological monitoring is already taking place, and these are often good candidate sites for an ARMS observatory when the purpose is long-term monitoring. Observatory sites placed in close vicinity to introduction hotspots, such as aquaculture facilities and ports or marinas, will help the detection of NIS and they should be easy to reach to take samples. There has to be a good chance that the ARMS will not be removed or disturbed by visitors and people working in the area. As well as asking for authorisation from the local authorities (e.g., MPA managers, port managers), according to your local or national rules, you should add contact labels to ropes and buoys (Fig. 1D) to minimise the risk of the ARMS being removed or disturbed. In marinas and ports, it may be a good practice to deploy the ARMS under the floating pontoons, where many of the non-indigenous species typically settle and contribute to the biofouling communities in these artificial habitats.

How much time should the ARMS be deployed? In temperate waters, we recommend that you deploy ARMS between 3 to 6 months *minimum* and preferably run a first trial to select the appropriate time frame. A few months allow for the detection of non-indigenous species for example. Depending on your region, deployments from spring to summer should be preferred. If you are planning to perform long-term monitoring, ARMS should be deployed for one year and can be retrieved while deploying the next one. One-year deployments will account for seasonality variations. For comparability, ARMS should be deployed every year in the same place.

Saving time and money. The main costs for maintaining an ARMS observatory are associated with deployment and retrieval. Some places require permits (e.g. ports, MPAs), which often take time to get. *We recommend that, during your design phase, you contact a partner who already runs an observatory of the same type as you would like to establish.* This way you can get help and advice, minimising the chance of unplanned obstacles.

Many partners deploy a new ARMS at the same time when they retrieve a submerged ARMS. This way you can save on expenses.

Diving is expensive, and you need to think about long-term costs for deployment/retrieval if you choose a site that needs scuba diving.

How many field replicates should be deployed? ARMS should be deployed in triplicates (three ARMS per site), in close proximity to each other, in the same habitat, at similar depth, and around the same dates. The guidelines are that between any two field replicate ARMS units, there should be:

- ≤ 10 m distance (preferably placed in a triangle)
- ≤ 2 m difference in depth
- ≤ 2 days difference in deployment time and retrieval time respectively

This will help to get a representative capture from the area, and robust data for later studies. See section 4.2 for more details on replicates, and their identifiers.

However, if you are not familiar with the area, you can start by deploying only one ARMS, to test the protocols, acquire practical experience, and explore the potential of a candidate site. For this, one ARMS unit would be sufficient during the first year, with replicates to be deployed in subsequent years.

Different sampling sites. Depending on the area you are monitoring, you might want to sample more than one location (e.g. ARMS units deployed at different sites within the study area) to represent different microhabitats and thus maximise the number of observed species/NIS. However, we highly recommend having 3 field replicates (see previous point) for each sampling site, increasing the number of ARMS needed in your observatory. First make sure that you have triplicates for your first sampling site before considering adding new ones. See section 4.3.

Practical details. ARMS can be attached to the jetties of marinas (e.g. underneath). In this case you should make sure that: i) the connecting rope is clearly marked with a label (Fig. 1D), and ii) the ARMS and connecting rope are not in the way of propellers or currents made by vessels and boats.

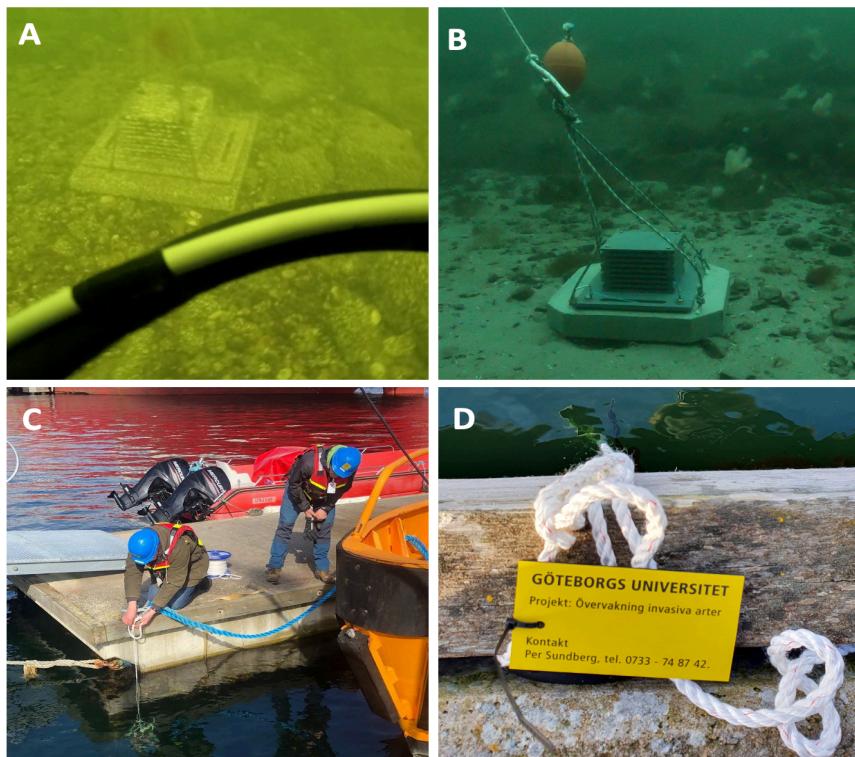


Figure 1.Examples of ARMS deployments at LTER sites with divers (A, B), in industrial ports (C), and marinas (D). In cases where ARMS are deployed with a rope and a buoy attached, please make sure you add a weather-proof contact label with your contact address.

3.2. Purchasing ARMS

ARMS should be purchased from the [Smithsonian Institution](#). They offer a good price for the units, and this also ensures that samples over the entire ARMS-MBON network will be comparable. Contact the ARMS-MBON network before ordering because we may organise large batch-orders during the winter months.

4. Identifiers used in ARMS-MBON

ARMS-MBON, and the wider EMO BON project, collect *a lot* of samples, and from those samples produce and collect *a lot* of data. All these data are shared, with the EMO BON team and with the wider world, and are eventually published in journals and public biodiversity archives. It is important that we produce data of quality and that they can be trusted, meaning that full traceability of scientific results to the data they came from must be made easy, for everyone and anyone. Hence, it is important that all material used, all material collected, and all data produced, receive unique, persistent, and correct identifiers (IDs).

4.1. Sample terminology

Using the right naming for samples is essential, because this will allow all partners in the network to organise and understand the data collected from the samples. Bear in mind that we will have 100s of individual datasets to organise and link to each other, each year of the project, and the IDs are the key to the combination of all of these data.

The IDs that we use, now that ARMS-MBON is part of EMO BON, are only slightly different to how they were before. All field data are collected by EMO BON in one log sheet (via google sheets, see detailed explanations [here](#)) per partner and per sample type (water, soft sediment, and the ARMS hard bottom), and an initial set of IDs will be already in your first set of log sheets to guide you in the future. In addition, one tab of each log sheet (called “definitions”) also explains how to format each entry of the log sheet.

We will use the following terms to describe and distinguish our material and data (Fig. 2):

1. **Observatory ID(s).** An observatory is an area in which ARMS units are placed. If the units are placed in very distinct areas (habitats) one may wish to define multiple observatories; both approaches are taken equally by the current set of ARMS-MBON partners. The observatory name should be short and unique: e.g. **Koster** for the Swedish observatory.
2. **ARMS unit IDs.** These IDs describe an ARMS unit located in a specific observatory. These IDs are not for the physical units but rather for the locations they are placed in; whether a unit is retrieved and then later replaced with a new unit *or* replaced with the old unit does not matter: the ID of whatever unit is in that place does not change. Unit names should be short and unique, they must be written as a continuous string that includes a name for the unit and a number for the replicate ([A-Z][1-9], no special language characters): for example, **VH1**, **VH2**, **VH3**. Once a unit ID has been chosen and samples (and then data) from it has been created, that ID must not be changed, otherwise we lose the history of that unit.
3. **Event IDs** are used to identify an event (which is deploying and then retrieving a unit). Event IDs for the ARMS events are constructed in the following way: [observatoryID]_[ARMSID][fieldReplicateNumber]_Ha_[deploymentDate]_[retrievalDate], where “Ha” stands for hard-bottom and the dates are formatted as **YYMMDD** (and not **YYYYMMDD**). For example **Koster_VH2_Ha_220415_220906**.
4. **Material sample IDs** are made up of the following
 - a. project (“EMOBON_”)
 - b. eventID
 - c. fraction: motile (_MF), sessile (_SF), or blank (_blank)
 - d. filter size in μm (unless blank sample): e.g. “40” for $40 \mu\text{m}$
 - e. replicate number: “_1/2/3/...”for example **EMOBON_Koster_VH2_Ha_220415_220906_SF40_1**.

This ID is also to be written on the material sample labels (see below for more detail). Under

ASSEMBLE Plus we did not regularly have technical sample replicates, but for EMO BON this is required.

The observatory and ARMS IDs are usually chosen when you join EMO BON and start with ARMS hard-bottom sampling. The IDs should be short, unique, and not include spaces, hashes, etc. Event IDs are unique to each event (=a unit has been retrieved and successfully processed). Material Sample IDs are unique to each material sample/replicate obtained from each event. These IDs are to be used in PlutoF (the data management platform we use.) and in the log sheets.

The IDs for the observatories and ARMS units that were part of ASSEMBLE Plus were chosen when that network was set up. When EMO BON took over, some of the same observatories and ARMS units transferred to the new organisation, and those observatory and unit IDs were/will be added to the observatory log sheets by the EMO BON secretariat and the observatory scientists. In Fig 2 (upper) the role of the different identifiers are outlined.

Observatory ID	ARMS ID	Sample type	Event ID	Material Sample ID
Koster	VH1	Ha	EMOBON_Koster_VH2_Ha_220418_220918_220906	EMOBON_Koster_VH2_Ha_220418_220906_SF40_1 EMOBON_Koster_VH2_Ha_220418_220906_SF40_2
Describes the location of the observatory where the ARMS are placed	A unique ID to each ARMS unit (specifically their location) in the observatory	Ha stands for hard bottom (Wa and So are for water and sediment)	Unique ID for the particular event for each unit	One material sample deriving from one ARMS unit from one sampling event. Adds sample information to the Event ID: sessile or motile fraction (SF, MF), filter size, replicate number (1, 2)

Figure 2. Table explaining how ARMS observatories, units, events, and samples should be named.

4.2. Replicates

Replicates are used to assess the reliability of the experimental procedure. But before getting into the definition of different levels of replication, we should identify the variable of interest. What we ultimately want to assess is not so much the community on the ARMS but the benthic community at the deployment locality. The ARMS unit itself is therefore part of the experimental procedure and should be considered as a standardised sampling device for the diversity of the local benthic community. We need several field and technical replicates for a reliable diversity assessment. With this consideration in mind, we define the following hierarchical structure of replicates within the ARMS programme:

1. Field replicates, i.e. separate ARMS units deployed very close to each other

Aim: here we have ARMS unit replicates in the sense of field replicates being deployed close to each other (ca. 3-10 m apart to avoid direct interaction) in a given locality and habitat over the same period of time. This should produce comparable results if there are no errors associated with their deployment, retrieval, and processing (scraping and homogenisation) in the laboratory. Such field replicates are important to estimate alpha diversity within, and beta diversity between, sites or habitats of interest. They are also crucial for replicability of the measurement, which is especially important for time-series in LTER research. We urge our partners to deploy **three ARMS per locality** (3 field replicates).

Careful! It is important to remember that field replicates must (1) be close to each other in location (10m linear separation distance max, 3m in depth max) and (2) they must be placed and retrieved at the same time (plus/minus 1-3 days, up to a week if the submerged time is longer than a month).

Important: Field replicates will be under the same **Observatory-ID** and **ARMS-ID**, and will be identified this way: “name1”, “name2”, “name3” (with “name” being the ARMS-ID and 1/2/3 being the number of the replicate)

Note: In previous years of the program, many observatories deployed ARMS in different locations of an area (previously called “independent replicates”). However, it is of more use for **robust science and statistics** if there are at least **three field replicates** in one single location. Please consider this for your next deployment and change your locations/ARMS-IDs accordingly.

Note: Be mindful when recording the coordinates of your ARMS, as this might influence their denomination. *Please give sufficiently precise coordinates to allow for a measurement of a metre: about 5 significant digits (1.12345 degrees).*

Note: If you have ARMS units in an observatory that are *field replicates* of each other, this is indicated in a dedicated column in your log sheet, and you will fill that in there. Since field replicates must not only be close to each other (in depth as well) but they should also be placed and retrieved within 1-3 days of each other, it is possible that a set of ARMS units will be replicates of each other in one event, but due to difficulties in retrieving the units they may not be replicates in another event. This needs to be correctly identified on the log sheets.

2. Sample replicates, or technical replicates, i.e. separate material samples from any ARMS unit during one sampling event

Aim: these material replicates are required to allow checking for sequencing artefacts and to obtain higher quality results. This is an adjustment to align with the EMO BON procedures, where the collected material from each ARMS unit is split into different containers, appropriately labelled, and shipped or biobanked.

As explained previously (see also Fig. 2), the *sample replicates* are included in the material sample IDs.

4.3. Sampling sites in one observatory

You might want to have ARMS deployed over a broader range in your observatory’s area (i.e. more than one sampling site). This can be useful to measure the different colonisation pressures from different benthic communities due to deployment of ARMS units at different sites and/or in different habitats.

However, as mentioned above, we highly recommend having **three field replicates per site**. Please consider new sites only if field replicates for your first site are secured.

Note: If you have more than one sampling site, they will be under the same **Observatory-ID**, but will have different **ARMS-IDs** that cannot be mixed up, and each have 3 field replicates (1/2/3) if possible.

5. Deployment and retrieval

For deployment and retrieval, ARMS-MBON methods are inspired by the standards and protocols established by the [Smithsonian Institution](#), but evolved as the project advanced. The Smithsonian protocols are not valid for ARMS-MBON any longer. Please follow the guidelines [in this handbook](#).

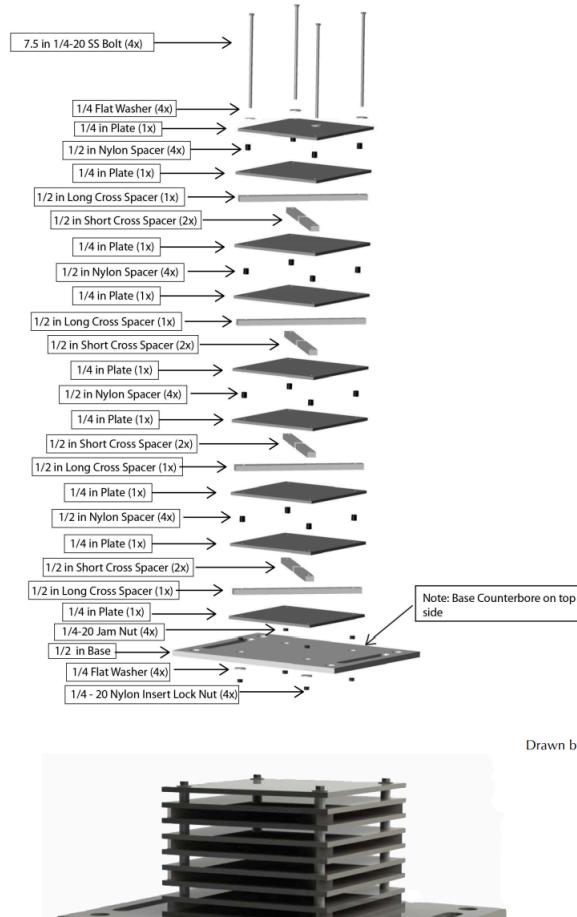
5.1. Assembly

→ To assemble your ARMS unit, follow this procedure (and see Figure 3 below):

Grease four bolt tips, place washers on each, and insert them through a plate's corners, flipping it so bolts point up. Add a nylon spacer and another plate on each bolt for an open layer. Use long cross spacers on two opposite bolts and short ones on the others, intersecting at right angles, with another plate for a closed layer.

Alternate open and closed layers until four of each are built. After the last layer, grease bolt threads, attach a washer and nut to each, and secure.

Place the base plate down, add washers and locknuts, and tighten. Invert the structure for completion.



Drawn by: D. Merritt



Figure 3. Illustration of ARMS unit assembly, source: Smithsonian Institute

5.2. Deployment

ARMS units should be placed and maintained on the substrate in a horizontal position. In many cases it is difficult to attach the ARMS to the sea floor. We recommend using commercially available concrete foundations and drill holes into them (Fig. 4A-B). We also suggest attaching an underwater buoy to the unit to facilitate its relocation. If the ARMS unit is standing on soft bottom, it is recommended that the foundation it is standing on puts it in a position >1 dm above the seafloor, to avoid the lower plates of the ARMS getting buried in sediment with time.

When the ARMS are secure on the substrate, take pictures of the ARMS and its surroundings, and mark a GPS point of the site. Record deployment metadata (date, location, depth, ARMS-ID) to later upload on EMO BON log sheets and create an event on PlutoF.

Note: As mentioned, attaching the ARMS to the sea floor can be challenging. You can use additional tricks like attaching weights to the sides of the base plate (without obstructing the water flow in the structure) or using zip ties to secure the ARMS on surrounding rocks, poles, etc.

5.3. Retrieval

Initial preparation. Use 10% bleach to sterilize the equipment to be used during retrieval: one water tub per ARMS unit, the crate cover, a water bucket, a 40µm filter and one air-stone per water tub. Rinse carefully with filtered seawater before use. On the boat or shore, filter sea water with the 40µm filter to fill up each clean tub. Avoid direct skin contact with the water to minimize introduction of exogenous DNA, use gloves and change regularly. Place an operating bubbler air-stone in every tub of water.

Cover container. To capture all motile and epi/hyper benthic fauna on the ARMS, we recommend putting a plastic container over the ARMS before retrieval. The container can be secured with rubber ropes to ensure it stays attached to the ARMS on the way to the surface (see Fig. 4C). Openings can be drilled to the sides of the container and lined with 40 µm mesh to allow for partial drain of excess water above the surface to facilitate transportation to the lab, while preventing the smaller portion of epifauna from escaping. You will be able to indicate on your log sheet whether you used a crate cover during retrieval.

Bringing the ARMS to the surface. Remove the anchoring system. Slowly bring the ARMS up, invert the unit so that the base plate is facing upwards. If the crate cover has a 40µm mesh, allow the water to drain off through it. Otherwise, try to transfer as much of the water from the crate as possible into the aerated tub without filtering it, but also without including too much sediment. Put the ARMS unit in the clean aerated tub and make sure all plates are submerged. Process the unit as soon as possible.

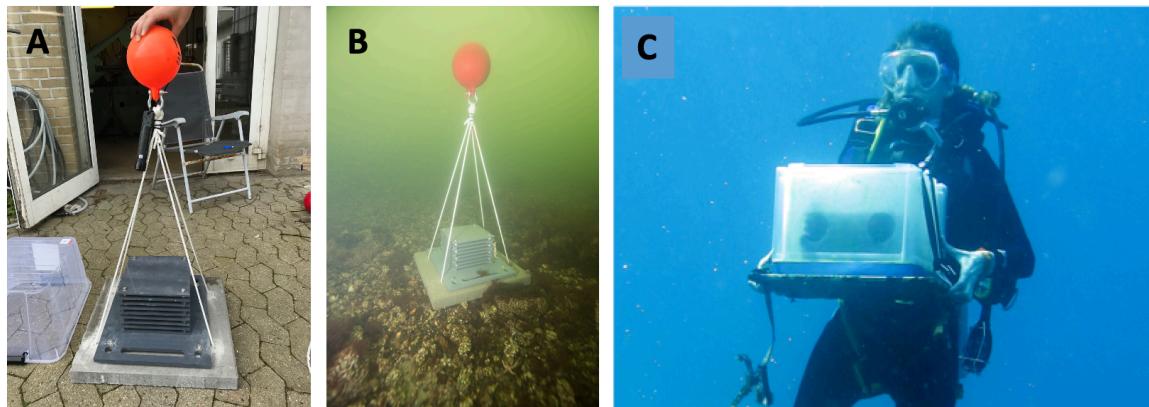


Figure 4. A-B. Photograph showing ARMS deployment mounted on a commercially available base plate. An underwater buoy is attached to help relocate the ARMS. C. Photograph of the plastic container that is put over the ARMS on the seafloor before retrieval. Photograph credits A-B: ARMS-MBON network. C: <https://www.oceanarms.org/>

6. ARMS processing

For ARMS processing, ARMS-MBON methods are inspired by the standards and protocols established by the [Smithsonian Institution](#), but evolved as the project advanced. The Smithsonian protocols are not valid for ARMS-MBON any longer. Please follow the guidelines [in this handbook](#).

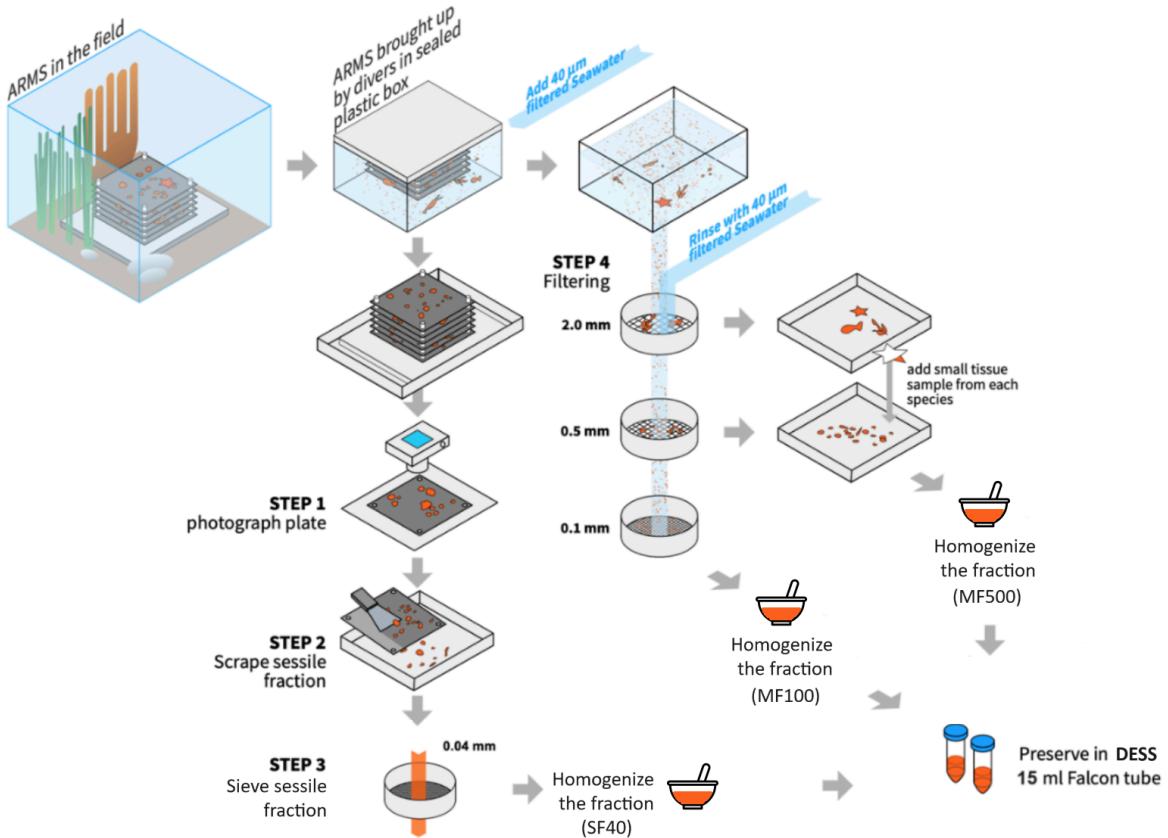


Figure 5. Schematic illustration of sample processing in ARMS-MBON, revised diagram from the Smithsonian protocols.

6.1. Disassembly

IMPORTANT: Prior to disassembly, all the containers, surfaces and tools should be meticulously cleaned with 10% bleach and then rinsed. This disinfection should also be done between processing of different ARMS units. Wear gloves at all times; do not touch the water with bare skin.

Remove the crate cover. Detach the crate and carefully lift it off the ARMS. Rinse all sides of the crate with filtered sea water, back into the ARMS recovery tub. Put the crate aside.

Unstack ARMS plates. During this procedure, make sure the plates do not spend too much time outside of the seawater. Using wrenches, unscrew the nylon-insert lock nuts from the base plate and put aside. Lift the base plate off the ARMS, and gently move it in the seawater tub to keep all motile organisms in. The base plate can be set aside at this point as it is not part of the ARMS unit and should not be processed any further. Unscrew the nuts at the base of the first plate and discard them. Lift the first plate (the plate closest to the base plate is the first place). Be careful to remember which side is the top of the plate. If the plate contains the spacers (short and long), remove them and shake them inside the water tub to remove motile organisms. Keep them in another bucket.

Note: The base plate is Plate 0. Then, from the closest plate to the furthest one, they are numbered from 1 to 9.

Store the plates before the next step. Stack the plates one by one in another bucket filled with aerated filtered seawater. Keep the same order and direction as removed (e.g. on a dish tray, see Figure 6), keep track of the number of the plate and the top and bottom sides.



Figure 6. Image of dismantled plates, stacked in the right order before further processing. Photo credits: <https://www.oceanarms.org/>.

6.2. Photographing

From each ARMS unit retrieved, you should take high-resolution images (see Fig. 8 for examples) of

- the plates (**mandatory**, [see procedure below](#))
- specimens from the plates (*optional*, Fig.8C)
- the habitat and surrounding environment (*optional*)
- the sampling event (*optional*)

Reminder! During manipulation of the plates, all the containers, surfaces and tools have to be bleached and rinsed. Wear gloves at all times; do not touch the water with bare skin.

Photography set up. Use a clean tray and fill it with filtered seawater. Put 4 clean nylon spacers to support the plates in the water. A stable external light source coming from above the tray should be placed so that light reflections can be avoided.

Camera technicalities. Do not use a wide angle as it distorts the image. Do not use flash not to create reflection in the water. Make sure that pictures are taken on a horizontal plane, to do so we strongly suggest the use of a camera tripod at a fixed distance (same for all plates for consistency). Record the camera settings you use.

Photography procedure. Place the first plate in the centre of the tray, it should be completely submerged in the water. Place a label on the plate in a way that it can be read from the image. Plate label templates can be found on the ARMS-MBON Github site (https://github.com/arms-mbon/documentation/tree/main/data_entry_templates). Take a photo of the overall plate, before removing the label and photographing more closely each corner and the centre. We suggest 3x2 (6) or 3x3 (9, see Figure 7) pictures per plate side, depending on the resolution obtained with your camera (more pictures needed if lower quality). Close-ups should have some overlap to allow later stitching of images. Repeat the whole procedure for the bottom side of the plate, using the corresponding label. Repeat for each plate.

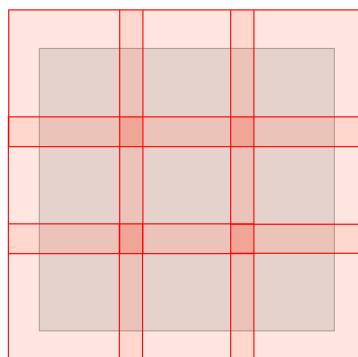


Figure 7. Schematic view of the grid that should be used for taking close-ups pictures. There should be some overlap between the close-ups so that they can be stitched together to obtain a high resolution overview of the plate.

Next step. For digital processing and management of your pictures, see section 7.1.

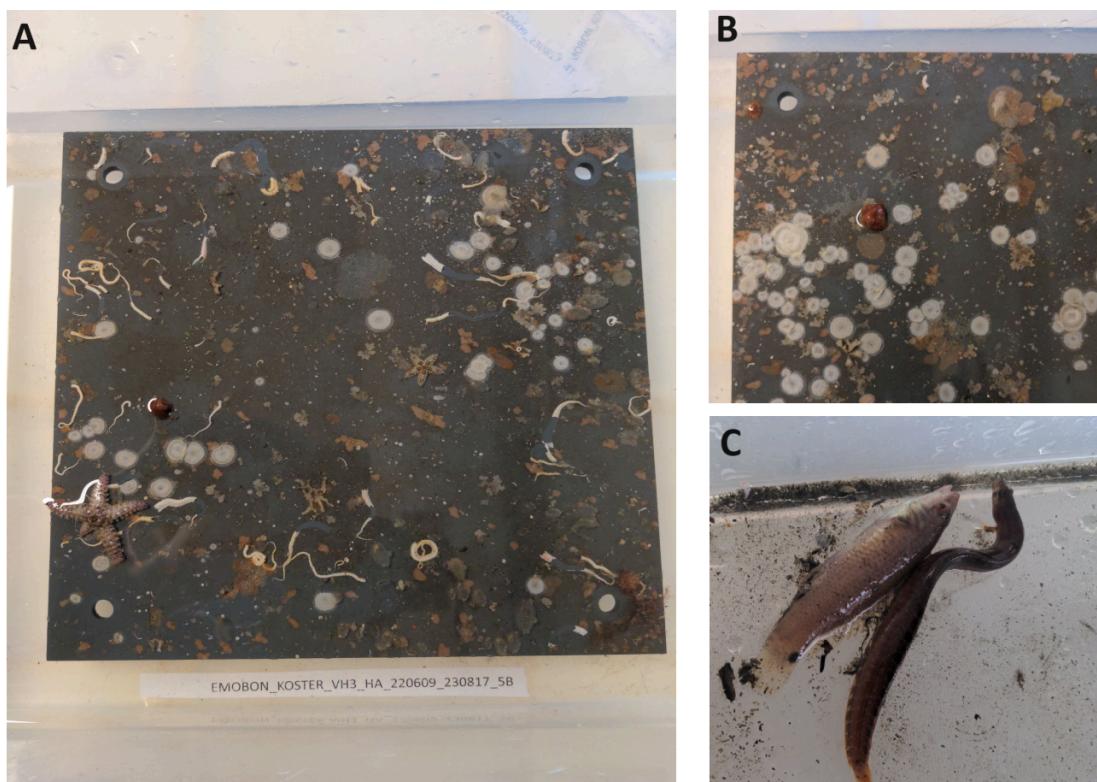


Figure 8. Examples of images of plates overview (A), close-ups (B), and isolated specimens (C), including labelling. Photograph credits: ARMS-MBON network.

When downloading the photos from the camera to the computer, they will have names as given by the camera software. Take note that it will be necessary to either rename these images before uploading to PlutoF, or to note down in a spreadsheet which unit and plate each image is of, including information about repeat images, zooms, or grid numbers. *It will be much easier for you to do this if you have kept track of your photos while you are taking them.*

6.3. Manual observations

In many cases partners make manual observations of species, for example during the ARMS retrieval or the sample processing. These are valuable records that can be added to the images and the sequences. As with the images, these will become part of the legacy of ARMS-MBON and will be available for others to use. It is therefore important to link these observations to the environment where they were recorded.

For digital processing and management of your manual observations, see section 7.2.

6.4. Vouchering

In ARMS-MBON, and as part of EMO BON, vouchering of organisms is not required. Collecting and storing organisms you find interesting is always encouraged, but ARMS-MBON/EMO BON does not currently provide the infrastructure to handle these.

6.5. Collection of fractions (Material Samples)

From each ARMS you should collect three size fractions (material samples):

- Motile fraction sieved with 500 µm (**MF500**)
- Motile fraction sieved with 100 µm (**MF100**)
- Sessile fraction sieved with 40 µm (**SF40**)

6.5.a. Motile fractions (MF100 and MF500)

→ During this step, the motile organisms from the ARMS disassembly water tub will be separated into two size fractions: the “100µm - 500µm fraction” and the “>500µm fraction”.

Reminder! During manipulation of the plates, all the containers, surfaces and tools have to be bleached and rinsed. Wear gloves at all times; do not touch the water with bare skin.

Sieving procedure. Pile up the 2mm, the 500µm and 100µm sieves and slowly transfer the water from the ARMS tub. Water AND sediment must pass through the sieves, you can clean the tub with filtered sea water and make this pass through the sieves. Make sure that all the water passes through before refilling the sieves or it will overflow. At the end, let as much water as possible drain off before moving the sieves.

Homogenization. Transfer the content of the 500-µm sieve (with as little water as possible) into a clean mortar. Add a few pieces found in the 2 mm sieve into the mortar. Grind until the obtention of a homogenous paste. Transfer the content of the 100-µm sieve (with as little water as possible) into a clean mortar and grind until the obtention of a homogenous paste.

Sample preparation. Divide the content of each mortar into several 15mL Falcon tubes. You should have up to 2mL of content per tube. Fill with DESS (see section 6.7) and shake vigorously to homogenise. Label the tubes and close with parafilm, place them at -20°C until shipping.

At least 4 technical replicates for each fraction should be collected from each sampling site. In case the material is not enough to collect 4 technical replicates, collect as many as possible; make sure to indicate the number of collected replicates in the metadata.

Labelling and storage. Label the tubes with the material sample ID (Sec. 4.1), cap the tubes tightly and seal with parafilm. Please use a printed label (you will find a template in our [GitHub space](#)) and NOT handwriting, which can be accidentally erased. Every sample tube should have its own individual label firmly taped around it. Place them at **-20°C** until shipping.

We recommend that you ship three technical replicates of each fraction (material sample, see section 8.2) and keep the remaining tubes as “backup-replicate” in long-term storage at your institution (see Sec. 9). Please remember to label these backups in the same way as the primary tubes.

Important: How to deal with large biomass or sediment?

Overcrowding with a single or a few species (see example in Figure 9) can lead to bias in the amplification of the DNA. This may increase the chance of missing a rare species during the molecular genetic processing, and clearly, we want to avoid this! One way to reduce the tissue bias is to include only a small proportion of the crowding species in your homogenisation, although it must be noted that this can similarly increase the chance of missing out a rare species in the actual sample processing. We recommend homogenisation of all tissue **ONLY** if you expect a NIS or a rare species among (or associated with) the crowding species. If that is not the case, you should reduce the biomass of the crowding species that is homogenised and processed. Appropriate notes should be included with the metadata for each ARMS that approximates the biomass (g) of the removed dominant species.

Sometimes ARMS are heavily covered with sand and silt that can create large sample volumes. In these cases, you need to separate the sand/silt from the organic material before you separate the different fractions by shaking the sample and decanting the organic suspension right after the sand/silt has sedimented. Thereafter you can filter the different fractions (i.e. 100, 500 micrometre).



Figure 9. Overcrowding of the ARMS surface with one or a few dominating species. Photograph credits: ARMS-MBON network.

6.5.b. Sessile fraction (SF40)

→ During this step, all sessile communities will be bulk collected from all the ARMS plates and sieved.

Reminder! During manipulation of the plates, all the containers, surfaces and tools have to be bleached and rinsed. Wear gloves at all times; do not touch the water with bare skin.

Scraping procedure. Before scraping, check if you have overcrowding species (see above), and if you do, manually discard those organisms. Then, place the first plate in a clean tray and scrape all sides (including the fine borders) into the tray. If needed, use the squeeze bottle to quickly rinse off the plate with filtered seawater. Only use a small quantity of water. Repeat for the rest of the plates (all scraped in the same tray).

Sieving procedure. Once you have all your content in the tray, pass everything through piled up 2mm and 40µm sieves. Water AND sediment must pass through the sieves, you can clean the tray with filtered sea water and make this pass through the sieves. Make sure that all the water passes through before refilling the sieves or it will overflow. At the end, let as much water as possible drain off before moving the sieves.

Homogenization. Transfer the content of the 40µm sieve (with as little water as possible) into a clean mortar. Grind until the obtention of a homogenous paste.

Sample preparation. Divide the content of the mortar equally into several 15mL Falcon tubes. You should have around 2mL of content per tube maximum. Fill with DESS (see section 6.7) and shake vigorously to homogenise.

Labelling and storage. Label the tubes with the material sample ID (Sec. 4.1), cap the tubes tightly and seal with parafilm. Please use a printed label (you will find a template in [this Github page](#)) and NOT handwriting, which can be accidentally erased. Every sample tube should have its own individual label firmly taped around it.. Place them at -20°C until shipping.

At least 4 technical replicates for each fraction should be collected from each sampling site. In case the material is not enough to collect 4 technical replicates, collect as many as possible; make sure to indicate the number of collected replicates in the metadata.

We recommend that you ship three technical replicates of each fraction (material sample, see section 8.2) and keep the remaining tubes as “backup-replicate” in long-term storage at your institution (see Sec. 9). Please remember to label these backups in the same way as the primary tubes.

6.6. Blank samples

Blank samples are essential to control for contamination from the sampling- and processing environment, and to verify the integrity of the experimental setup. Such samples can be obtained by using a cotton swab to wipe the surfaces involved in the collection of each fraction, prior to starting the processing. This swab is then preserved and stored along with the biological samples, i.e. in a 15mL Falcon tube filled with DESS from the same batch at -20°C. It is recommended to make 3-4 replicates of the blanks as well – be sure to wipe about the same spots to ensure comparability between the replicates.

By doing this, these blank samples help detect any extraneous DNA that may have been introduced during sample collection, thereby safeguarding the accuracy of your findings. See section 4.1 for terminology of blank samples.

6.7. Preservation

ARMS-MBON preserves all biological samples in **DESS**, which is a DMSO-based preservative. The recommended recipe is DMSO salt-saturated buffer (20% DMSO, 0.25 M EDTA, pH 7.5, NaCl saturated), as described by Seutin et al. (1991) (<https://cdnsciencepub.com/doi/10.1139/z91-013>). It is recommended to saturate the DMSO with salt **overnight**. Please be clear when mentioning the preservative used.

In the first years of the programme, we also used EtOH and pure DMSO in some cases, and that is why you may see this indicated in some of the early ARMS sample data.



Figure 10. Labelled samples from one retrieval event: 3 ARMS units (field replicates) * 3 fractions * 4 technical replicates = 36 tubes. In addition, 3 technical replicates of the blank sampling of the processing station (purple caps).. Credits: ARMS-MBON

7. Digital processing of ARMS data

7.1. Image data

All collected images should be uploaded to the data management platform, [PlutoF](#), for each sampling event they belong to.

As images are digital data that will be shared via the ARMS GitHub site and scientific and data publications, it is necessary that the images are adequately described with metadata that explain what the images are of. To do so, when uploading your images on PlutoF, you need to upload a descriptive spreadsheet along with your images, containing their metadata. That spreadsheet should include descriptions of all the images you have uploaded for that event. It is **required** that the name of this spreadsheet uses the **Event ID** (see section 4.1) followed by “_images.csv”, for example **EMOBON_Koster_VH2_220418_220906_images.csv**. It is only with the presence of a file of this name that we know that you have provided this spreadsheet, rather than one with other information in it. Into this spreadsheet you will enter the image file names (i.e. the names of the file uploaded to PlutoF), the event ID, the image type (field, plate, etc), the plate number/face or field/voucher specimen information, and additional information (for example if the image is a zoom or of a gridded part of the plate). A template of this spreadsheet can be found on the [ARMS-MBON GitHub site](#).

At present, nothing about the images needs to be added to the EMO BON log sheets, other than a column in which you indicate that the images have been uploaded to PlutoF.

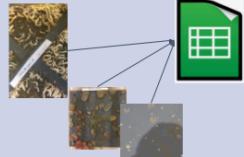
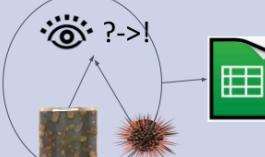
Image description spreadsheet	Manual Observations spreadsheet
EMOBON_Koster_VH2_Ha_220418_220906_image s.csv	EMOBON_Koster_VH2_220418_220906_ManualObserva tions
The filename of the spreadsheet accompanying images to describe them. Template of this can be found on the ARMS-MBON GitHub and will include the plate number (0-9) and plate face (Top and Bottom)	The filename to use for the spreadsheet with manual observations of images or done in the field or lab, for each sampling event
	

Figure 11. Table explaining how the Image Description spreadsheet and the Manual Observation spreadsheet should be organised.

7.2. Manual observations

The species identified from these observations are part of the ARMS-MBON data package for each of your events, and hence the relationship of these observations to the rest of your data needs to be clear, so that the following questions are clear to the user of the data:

- From where did you make your observation – an ARMS plate while you were processing it, an ARMS plate photograph you took, something motile in the water as you retrieved your unit, etc?
 - Note here that eyeball inspection of images is not the same as eyeball inspection of actual plates: the difference is not irrelevant, as the source data in the first case are digital image files (which may later be analysed by someone else), while the source data in the second case is your eyeball—brain (and no-one will be able to redo that later).
- Is this part of the sessile or motile fraction you will process and ship, or not? This is important because we need to know whether what you see in your manual observations could also appear in the DNA.
- Details of the observation itself: species ID, quantity, etc.

Manual observations should be made following a template that is provided on the [ARMS-MBON GitHub site](#). Please fill in all the mandatory fields here. Your manual observations can be uploaded to the associated data page for your event, with the following filename, which is also the **Manual Observations ID**

- EventID + “_ManualObservations.csv”,
e.g. [EMOBON_Koster_VH2_220418_220906_ManualObservations.csv](#)

This should be uploaded along with your images in PlutoF; in the case that you upload more than one such spreadsheet, simply add an iterator “_2” to the filename.

The IDs for the manual observations’ spreadsheet are highlighted in Fig. 11.

8. Shipment

8.1. Shipping times and address

Under EMO BON material is shipped to Paris at regular intervals, a few times a year. All EMO BON partners will be informed by the EMO BON secretariat when the next shipping will be. Until that point, you are requested to keep the ARMS samples in a freezer in your local biobanking facility. Please pay attention to the labelling of the shipped material: the material sample ID should be written on the sample containers as well as with the paperwork sent with them. Please use a printed label (you will find a template in our [GitHub space](#)) and NOT handwriting, which can be accidentally erased.

The requirements for dealing with permits for ARMS sampling are changing now we are part of EMO BON, but the details have not yet been settled. If you have obtained permits relevant to material samples you eventually ship to Paris (EMO BON HQ), please include this with your shipped material.

Note that shipment procedures have changed since the last version of the Handbook. All ARMS sample shipments are programmed to take place once a year in September and early October. All participating observatories will be notified by email on the specific dates, shipment address and any relevant details.



Figure 12. Samples ready for shipment. Photograph credits: ARMS-MBON network.

8.2. Checklist for the sample package

The following items need to be in the sample package:

- **For stations that are part of EMO BON (PiE, Koster, VLIZ, SBR, HCMR, Eilat, Getxo, Vigo, TZS):**
 1. Three 15mL tubes per ARMS unit successfully retrieved, i.e. one sessile and two motile fractions, and with labels as explained above. Ideally you will also include up to two technical replicates for each fraction, and a blank sample. We recommend that you keep the remaining, labelled, falcon tubes as “backup-replicates” for long-term storage in a freezer in your institute.

(For these stations, ABS has been taken care of: EMO BON biological resources are compliant with applicable national legislations - ABS and UNCLOS - in those countries, and therefore, samples can be sent without any further document, information etc.)

- **For other stations:**
 1. Three 15mL tubes per ARMS unit successfully retrieved, i.e. one sessile and two motile fractions, and with labels as explained above. Ideally you will also include up to two technical replicates for each fraction, and a blank sample. We recommend that you keep the remaining, labelled, falcon tubes as “backup-replicates” for long-term storage in a freezer in your institute.
 2. Filled out, printed and signed [Material Transfer Agreement](#)
 3. ABS declaration of due diligence. See how to do this [here](#) and [here](#).

Please keep copies of all documents together with the “backup-replicate” samples in your institution.

8.3. What happens next?

Once samples arrive at [EMBRC Headquarters](#), you will receive a confirmation email (if not, please send a reminder 2 weeks after shipping your samples). The samples will be processed as a batch at a centralised sequencing centre approximately 2-3 months after they are received. We sequence the genetic markers *COI* and *18S rRNA* (V1-V2 region) following the [EMO BON Molecular Standard Operating Procedures](#). Once the sequences are produced, they will be uploaded to European Nucleotide Archive (ENA) and the run accession numbers will be added to the EMO BON documents on the EMO BON GitHub space. All sample providers will have exclusive access to the sequences for a moratorium period of 6 months. Thereafter these sequences will automatically be made public.

In addition, we will periodically run a sequence cleaning, trimming, and analysis for all raw sequences using the [PEMA pipeline](#) to generate a consistent data product from the raw sequence data. These data will likewise be made available to you through the ARMS-MBON GitHub pages.

Each year, all data in PlutoF will be linked to a metadata record in IMIS; for more detail see the [Data Management Plan](#).

9. Biobanking

Two of the technical replicates sent will be processed for the extraction of DNA and subsequently sequencing. Additionally, 1 technical replicate per sample will be provided as a service from EMBRC to external users. We ask all partners to keep the remaining samples (at least one) as “backup-sample” replicate from each of the three fractions of one ARMS unit during a sampling event, together with a copy of the legal documents when applicable (see section 7.2, ABS IRCC or due diligence - as explained [here](#) and [here](#)), as well as a digital copy of all original images from the sampling event and from the processed plates. Please mark the samples as described in Sec. 8.1 and place them in a long-term storage freezer at -20 °C or colder in your institute.

10. Data management

The initial data management plan of ARMS-MBON can be found on the [ARMS-MBON GitHub](#) pages. This is now merged with the [EMO BON DMP](#).

Observatory and sampling event information for all EMO BON events are recorded by the sampling scientists in their own (institute-specific) EMO BON log sheets, and these will form the prime source of all EMO BON metadata that will eventually be combined, semantically annotated, and shared. More information about the structure of these log sheets can be found on [this GitHub page](#).

The ARMS-MBON sampling also uses the PlutoF data management platform to store the images and spreadsheets (images metadata and manual observations) from each sampling event, and for that you necessarily need to add some observatory and event metadata to PlutoF. A guide to using PlutoF for ARMS-MBON is described in this [GitHub](#) folder.

On regular occasions, the metadata in the log sheets and the metadata and data files in PlutoF are downloaded to GitHub to be quality controlled, combined, and shared. This part of the data management is still in progress, and we will give you more information here when that is ready.

11. Contacts

Matthias Obst, ARMS-MBON coordinator: matthias.obst@marine.gu.se

12. Links

The following documentation can be found on the ARMS-MBON [GitHub documentation repository](#)

- Data Management Plan
- PlutoF user guide
- Standard Operating Procedures taken from the Global ARMS Smithsonian site
- Molecular SOPs
- Access and Benefit Sharing guides
- This Handbook

The following templates can be found on the ARMS-MBON [GitHub template repository](#)

- Describing your photographs
- Creating manual observation spreadsheets
- How to create your various IDs
- Sample labels
- Material transfer agreement template
- Plate label template

Additional links

- The ARMS-MBON space on the data management platform PlutoF: <https://plutof.ut.ee/#/study/view/81139>
 - EMO BON website: <https://www.embrc.eu/emo-bon>
 - EMO BON Handbook: https://www.embrc.eu/sites/default/files/publications/2024.04_EMOBON%20Handbook_FINAL.pdf
 - EMO BON DMP: <https://repository.oceanbestpractices.org/handle/11329/1918>
 - EMO BON metadata logsheets templates: <https://www.embrc.eu/emo-bon>
-

13. Checklist

Important things to remember when performing your ARMS-MBON activities

- Stick to the observatory and ARMS unit IDs as chosen in the beginning.
- Review how to create event and sample IDs (Sec. 4.1) and how to describe your images and manual observation files (Secs 7.1, 7.2). It is important not to make mistakes in these IDs.
- Create your Observatory and ARMS unit site pages in PlutoF as soon as they are known, using the correct IDs. A guide to using PlutoF for ARMS-MBON is described in this [GitHub](#) folder.
- Create sampling events for each ARMS unit you manage in PlutoF after you have deployed your unit, and then to update it when you have retrieved your unit.
- Create an image description spreadsheet and upload that to PlutoF along with your images to your event page. Use the provided templates (Secs 7.1, 7.2, and see the PlutoF guide).
- List all the material samples collected and to complete all their metadata in the dedicated log sheets provided to you.