Handbook

for

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

Version

2

Date

2023-11-14

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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 in the followed procedures: while the sampling procedures remain the same, the IDs for events and samples, and the shipping and sequencing steps, have all changed. 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; sample processing, 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, e.g. about 3 months for the monitoring of Non-Indigenous Species (NIS) in marine coastal environments, and 1-2 years for Long-term Ecological Research (LTER) sites to study the status and changes in hard-bottom species communities.

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. Clarification on the definition of field replicates (section 4.2)
2. Clarification on the sample terminology (section 4.1)
3. Updated instructions for labelling and preservation (section 6)
4. Detailed instructions for EMOBON log sheets added

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 sent an email to: Matthias Obst (ARMS-MBON coordinator) matthias.obst@marine.gu.se, or Ioulia Santi (EMO BON secretariat): ioulia.santi@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. Currently, ARMS-MBON supports two kinds of investigations:

i) Non-indigenous species (NIS) surveys and monitoring, based on short-term deployments during summer season in presumed introduction hotspots (see below)
ii) Biological monitoring, based on long-term deployments for 1-2 years (i.e., Long-term ecological research; LTER)

For both investigations 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 NIS or LTER 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 LTER monitoring.

For NIS monitoring, observatory sites should be placed in close vicinity to introduction hotspots, such as aquaculture facilities and ports or marinas, 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 NIS typically settle and contribute to the biofouling communities in these artificial habitats. In temperate waters, we recommend that you deploy ARMS for a short period, not exceeding 3 months, and preferably run a first trial to select the appropriate time frame. Depending on your region, spring to summer should be preferred. We also recommend you deploy your ARMS 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 (10 metres maximum). This will help to get a representative capture from the area, and robust data for later studies. However, many partners 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 more ARMS to be deployed in subsequent years. See later for more on replicates and their identifiers.

Spatio-temporal distribution of field replicates. For NIS monitoring, it is good practice to have independent replicates (ARMS units deployed at different sites within the study area, e.g. the marina that is being monitored) to represent different microhabitats and thus maximising the number of

observed NIS. However each site would ideally need 3 field replicates, increasing the number of ARMS needed. For LTER the number of samples is less important, however for time-series the replicability is crucial: we recommend selecting one specific site within the area of interest, and deploying three ARMS in close vicinity within the same habitat: these would be field replicates.

Practical details. Especially for NIS studies, ARMS can be attached to the jetties of marinas (e.g. underneath). In this case you have to 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.

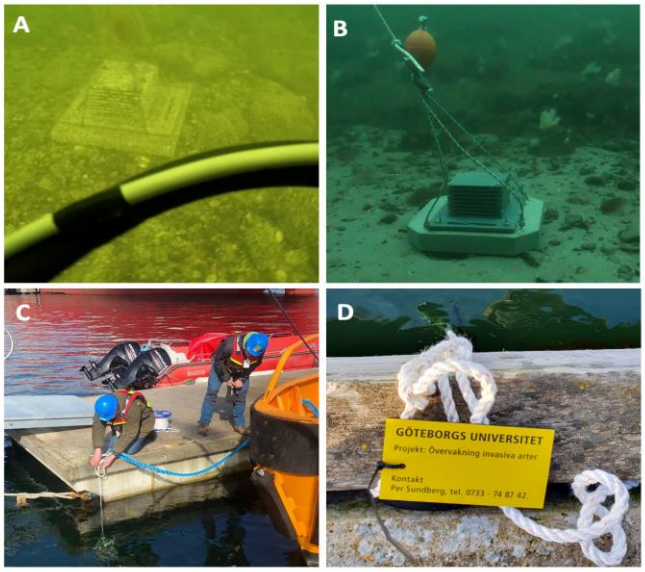


Fig 1. *Examples of ARMS deployments at LTER sites with divers (A, B), in industrial ports (C), and marinas (D). In cases where ARMS are deployed with a rope and a buoy attached, please make sure you add a weather-proof contact label with your contact address and telephone number (D). Photograph credits: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

3.3. 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 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 sweet and unique. 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.
   It is highly recommended to have three field replicates (see section 4.2 for guidelines about replicates). In that case the IDs would contain the number of the replicate, like this: "name1", "name2", "name3".
3. **Event IDs** are used to identify an event (which is placing and then retrieving a unit). Event IDs for the ARMS events are constructed in the following way: EMOBON_[observatoryID]_[ARMSID]_Ha_datein_dateout, where "Ha" stands for hard-bottom and the dates are formatted as **YYMMDD** (and not YYYYMMDD). For example, EMOBON_Koster_VH2_Ha_220415_220906.
4. **Material sample IDs** are made up of the following
   a. eventID + "_"
   b. fraction: motile (MF) or sessile (SF)
   c. filter size (in microns) + "_": 100_ for 100 microns
   d. replicate number: "1/2/3" or "blank"

   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.

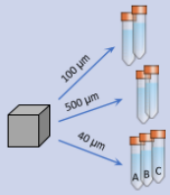
Observatory ID	ARMS ID	Sample type	Event ID	Material Sample ID
Koster	VH1	Ha	EMOBON_Koster_VH2_Ha_220418_220906	EMOBON_Koster_VH2_Ha_220418_220906_SF40_1 EMOBON_Koster_VH2_Ha_220418_220906_SF40_2
Describes the location of the observatory where the ARMS are placed	A unique ID to each ARMS unit (specifically 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)
				

Fig 2. Terminology explaining how ARMS observatories, units, events, and 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. **Independent replicates**, *i.e. several ARMS units deployed over a broad study area*

Aim: here we would have a measure of the different colonisation pressures from different benthic communities due to deployment of ARMS units at different sites and/or in different habitats. This provides an independent measurement of species diversity. Such "independent replicates" allow one to, for example, assess gamma diversity within the study area.
Important: Independent replicates will be under the same **Observatory-ID**, but will have different **ARMS-IDs** that cannot be mixed up.

2. **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. This means that each independent replicate (see above) should have its own field replicates. 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 recommend using three ARMS per locality.
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 their **ARMS-ID** will be structured this way: "name**1**", "name**2**", "name**3**" (with "name" being the same)

Note: Instead of deploying one or two ARMS in different locations (independent replicates), it is of more use for **robust science and statistics** if there are at least **three** field replicates in one single location.

Note: Be mindful when recording the coordinates of your ARMS, as this might influence their

denomination.

3. **Sample replicates or technical replicates**, *i.e. separate material samples from any one 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. 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.

5. Deployment and retrieval

5.1. General guidelines

For deployment and retrieval, ARMS-MBON follows the standards and protocols established by the Smithsonian Institution, with some additional guidelines. The Global ARMS protocols can be found on the Smithsonian website, but we have also copied the zip file to the ARMS GitHub site ARMS GitHub site where they will always be available.

5.2. Our additional guidelines

Base plate. In many cases it is difficult to attach the ARMS to the sea floor. We recommend using commercially-available base plates and drill holes into them (Fig. 3A-B).

Cover container. In order 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. 5). 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 or not you used a crate cover during retrieval.

Photographs. When taking photographs of the ARMS plates, please put a label on the plate so that it can be read from the image. Plate label templates can be found on (https://github.com/arms-mbon/documentation/tree/main/data_entry_templates).

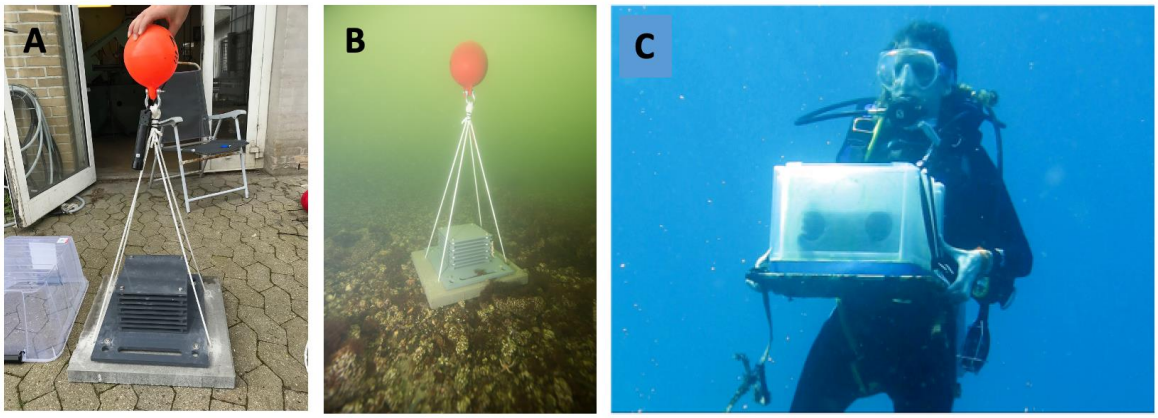


Fig 3. *Photograph showing ARMS deployment mounted on a commercially-available base plate (**A-B**). An underwater buoy is attached to help relocate the ARMS (**B**). **C.** Photograph of the plastic container that is put over the ARMS on the seafloor before retrieval (C). Photograph credits A-B: ARMS-MBON network. C:* https://www.oceanarms.org/.

6. Sample processing (physical and digital)

For all sample processing steps, ARMS-MBON follows the standards and protocols established by the Smithsonian Institution. The Global ARMS protocols can be found on the Smithsonian website, but we have also copied the zip file to the ARMS GitHub site (https://github.com/arms-mbon/documentation/tree/main/standard_operating_procedures) where they will always be available. *Please do read these carefully*: here we outline *only* the amendments and specific additions to these protocols that customise the ARMS for hard-bottom monitoring in European coastal waters.

6.1. 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 program, 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.

6.2. How to deal with large biomass or sediment

Overcrowding with a single or a few species 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/slit has sedimented. Thereafter you can filter the different fractions (i.e. 100, 500 micrometre).

Fig 4. *Overcrowding of the ARMS surface with one or a few dominating species. Photograph credits: ARMS-MBON network.*

6.3. Material Samples

From each ARMS you should collect three size fractions (material samples). All material samples should be **preserved in DESS**:

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

In many cases you will produce several tubes of 15mL from each material sample. **All** samples must be **homogenised** with a blender or mortar, and then kept in the **freezer** until shipping.

You should label these with the material sample ID (Sec. 4.1). Please use a printed label (you will find a template in our GitHub space) and NOT handwriting, which can be accidentally erased. Labels should be taped on top of **all** samples.

We recommend that you only ship two technical replicates of each fraction (material sample) and keep the remaining tubes as "backup-replicate" in long-term storage at your institution (see Sec. 7). Please remember to label these backups in the same way as the primary tubes.

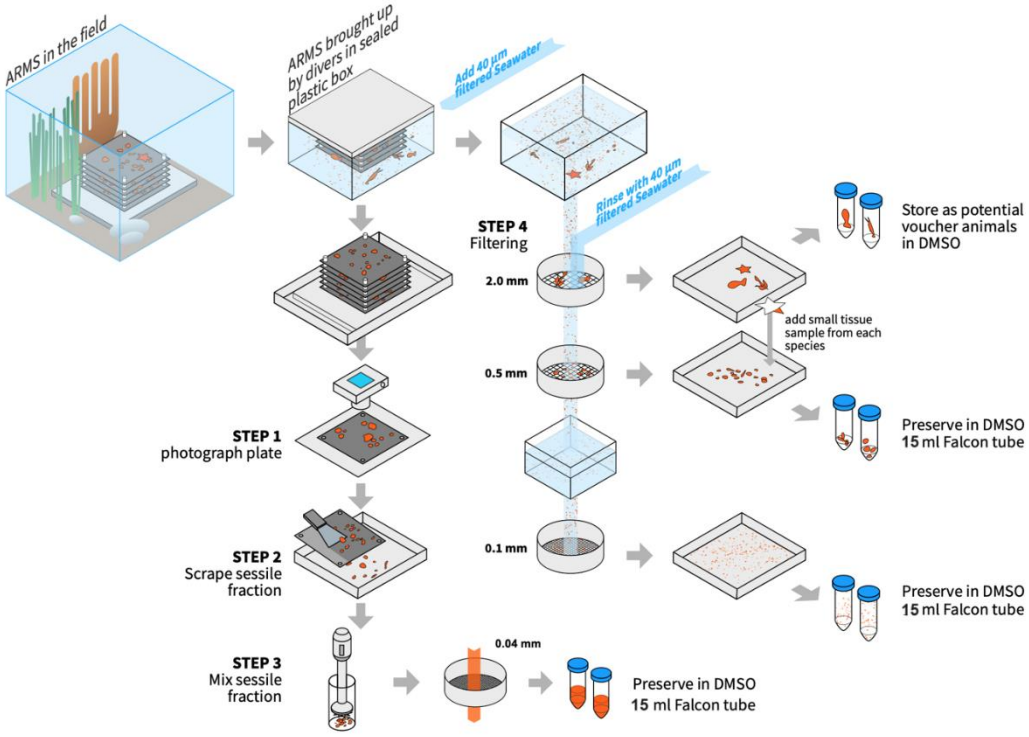


Fig 5. *Schematic illustration of sample processing in ARMS-MBON. For details see the protocols established by the [Smithsonian Institution](#).*

6.4. Images

From each ARMS unit retrieved, you should take high-resolution images (see Fig. 6 for examples) of

- the plates (mandatory)
- specimens from the plates (optional)
- the habitat and surrounding environment (optional)
- the sampling event (optional)

These 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, the event ID, the image type (field, plate, etc), the plate number/face or field/voucher specimen information (see Fig.7). A template of this spreadsheet can be found on the ARMS MBON GitHub site.

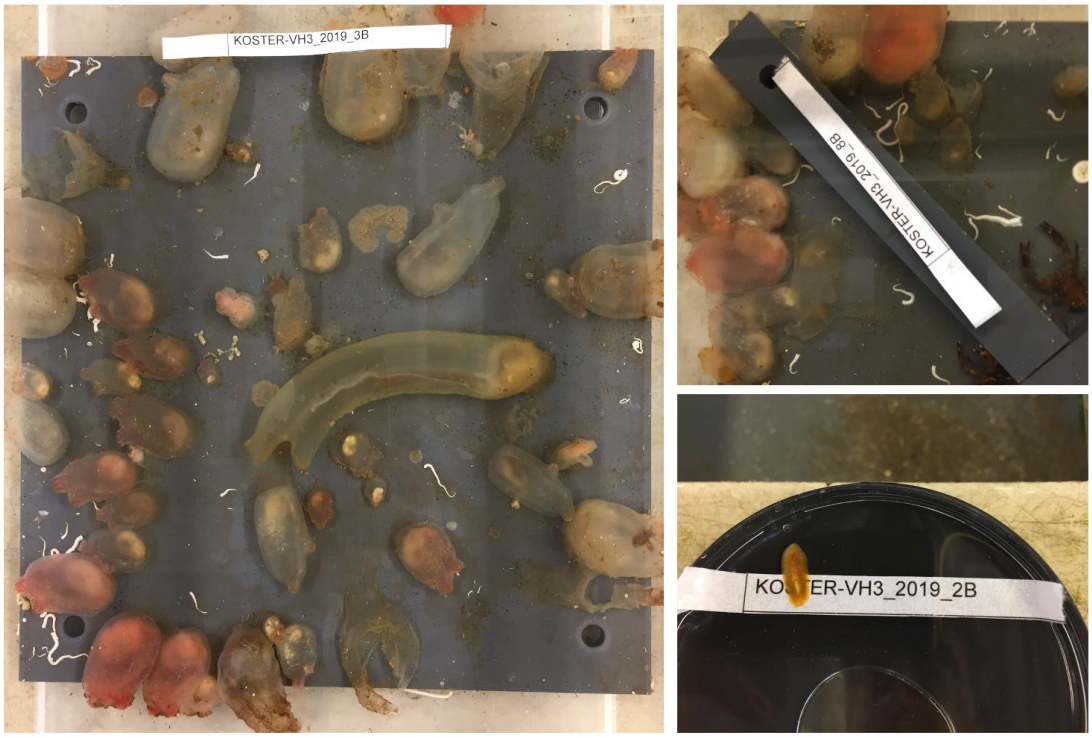


Fig 6. *Examples of images of plates, close-ups, and isolated specimens, including labelling. Photograph credits: ARMS-MBON network.*

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.

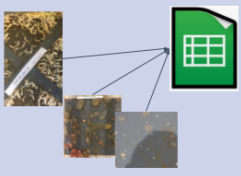
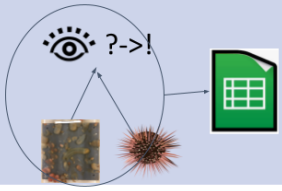
Image description spreadsheet	Manual Observations spreadsheet
EMOBON_Koster_VH2_Ha_220418_220906_images.csv	EMOBON_Koster_VH2_220418_220906_ManualObservations
The filename of the spreadsheet accompanying images to describe them. Template of this can be found on the ARMS-MBON GitHub and will include the 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
	

Fig 7. *Examples of images of plates, close-ups, and isolated specimens, including labelling. Photograph credits: ARMS-MBON network.*

6.5 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.

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. 7.

7. Shipment

7.1. 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 as outlined in Sec. 6.3: 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.

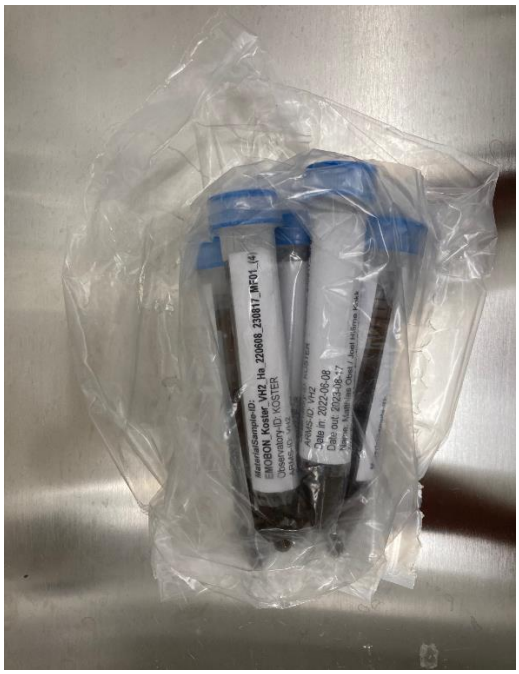


Fig 8. *Samples ready for shipment. Photograph credits: ARMS-MBON network.*

7.2. 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 and Vigo):

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. 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. 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.

7.3. 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*, *18S rRNA* (V1 region). 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.

8. Biobanking

We ask all partners to keep at least one "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. 7.1 and place them in a long-term storage freezer at -20 °C or colder in your institute.

9. 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 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.

10. Contacts

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11. 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://repository.oceanbestpractices.org/handle/11329/1738
- EMO BON DMP: https://repository.oceanbestpractices.org/handle/11329/1918
- EMO BON metadata logsheets templates: https://www.embrc.eu/emo-bon

12. Checklist

Important things to remember when performing your ARMS-MBON activities:

- Remember to 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 6.4, 6.5). It is important not to make mistakes in these IDs.
- Remember to 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.
- Remember to 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.
- Remember to create an image description spreadsheet and upload that to PlutoF along with your images to your event page. Use the provided templates (Sec. 6.4, 6.5, and see the PlutoF guide).
- Remember to list all the material samples collected and to complete all their metadata in the dedicated log sheets provided to you.