# Handbook

for

Marine Biodiversity Observation network for genetic monitoring of hard-bottom communities (ARMS-MBON)

Version

2.0

Date

2020-11-09

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| Date | Version | Change |
|---|---|---|
| 2020-04-01 | 1.0 | First version |
| 2020-11-09 | 2.0 | Sec. 2.4 (new); Sec. 2.5, 4.3, 4.4, (IDs); Sec. 2.6 (new); Sec. 4.5 (new, data template spreadsheet) |
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# 1. Summary

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). This document collects the guidelines, protocols, and recommendations for: obtaining, and then the deployment and retrieval of ARMS observatories as established and further developed by the network; site location choices; 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.

ARMS are highly standardised passive collectors for the assessment of epibenthic and hyperbenthic marine communities. In ARMS-MBON they are deployed 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.

# 2. Design your observatory

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

## 2.1. 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 prefered. We also recommend you deploy your ARMS every year in the same place.

**Definition of 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 measuring device for the diversity of the local benthic community. We need several 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 samples, i.e. several ARMS units deployed over a broad study area:* here we would have a measure of the different colonisation pressure 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.
2. *Technical or field replicate, i.e. separate ARMS units deployed very close to each other:* here we have ARMS replicates in the sense of technical replicates, being deployed close to each other (ca. 3-10 m apart to avoid direct interaction) in a given locality and habitat. 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. We recommend using three ARMS per locality.

Note that material sample replicates (Sec. 4.3) are separate to these ARMS (units) replicates.

**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 replicates should be deployed?** We recommend deploying three ARMS per site, in close proximity to each other. This will help to get a representative capture from the area (replicate type 2). 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.

**Spatio-temporal distribution of replicates.** For NIS monitoring, the three ARMS should be deployed as independent samples (replicate type 1), at different sites within the study area (e.g. the marina that is being monitored) which represent different microhabitats and thus maximising the number of observed NIS. 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 (type 2 in the list above).

**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.

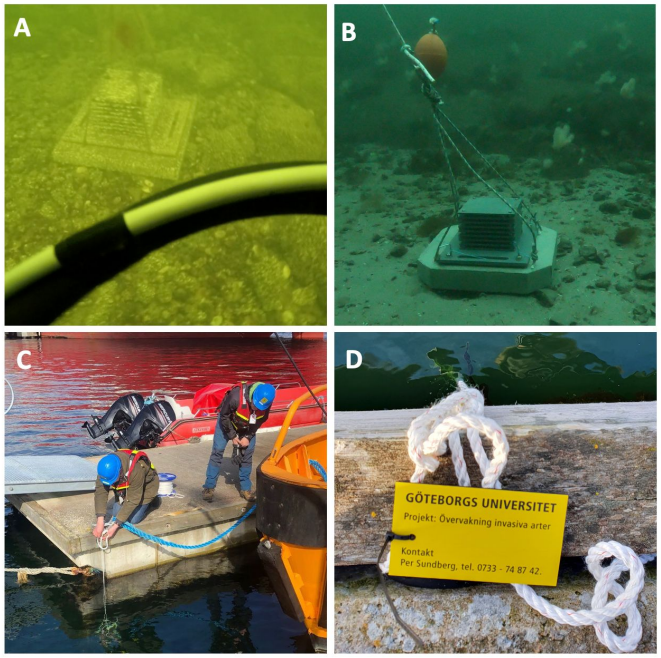



***Fig 1.*** *Examples of ARMS deployments at LTER sites with divers (A, B), in industrial ports (C), and marinas (D). In cases where ARMS are deployed with a rope and a buoy attached, please make sure you add a weather-proof contact label with your contact address and telephone number (D). Photograph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

## 2.2. Registration

We recommend that you start preparing for the deployment of the ARMS at your chosen observatory site(s) about 3-4 months ahead of time. You should register as a new partner of ARMS-MBON using the registration form available on the ARMS-MBON website (www.arms-mbon.eu). Once registered, you will be added to the ARMS-MBON email list and your observatory will be added to the ARMS-MBON database. As part of the registration process, you will be asked to read and comply with the Data Management Plan and supply some basic metadata for your observatory. We will then help you with the practical preparations, and discuss the specific design of your observatory with regard to purpose, location, number of ARMS replicates, and deployment periods.

## 2.3. Purchasing ARMS

ARMS should be purchased from the Smithsonian Institution (https://www.oceanarms.org/). 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 larger batch-orders during the winter months.

## 2.4. Once you have ARMS data to provide

Once you have run your first year of your ARMS units, and have data and metadata to provide (images, sample IDs, etc), you will need access to the PlutoF data management platform, and the overview google spreadsheet. You need to manage your (meta)data in PlutoF and the overview spreadsheet yourself. Consult the ARMS website for useful documentation and contact details.

## 2.5. Sample terminology

Here we explain the terms, the IDs, you need to use for all your ARMS data. It is important that all of these IDs are correctly used. All IDs should also be added to the ARMS data overview spreadsheet (sheets "ARMS Observatory info" and "ARMS metadata file") and in PlutoF. 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! Doing this properly will allow data taken in one year to be directly comparable to the data taken in any other year.

It is important to not change any IDs from year to year unless you substantially change the ARMS location; dates, depth, and latitude and longitude will be required metadata you will need to add to PlutoF, hence an observatory is distinguished not only by ID but also by geographic and temporal metadata.

| Sample region (country) | Observatory-ID | ARMS-ID | MasterSample-ID |
|---|---|---|---|
| North Sea Skagerrak (Sweden) | Koster | VH2 | ARMS_Koster_VH2 |
| Describes the region of deployment and the terretorial country | Describes the location of the observatory where the ARMS are placed | Assigns a unique ID to each ARMS in the observatory (i.e. spatial unique identifier) | Unique ID based on combination of previous IDs. Is the base ID for those from material samples, images, and manual observations |
|  |  |  | |

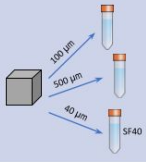
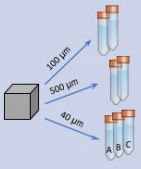
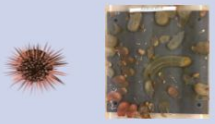
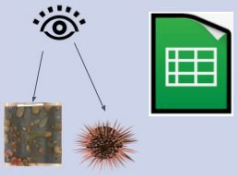
| Material Sample-ID (Fraction size) | MaterialSample-ID for replicates | Image IDs | ManualObservation IDs |
|---|---|---|---|
| ARMS_Koster_VH2_180418–180906_ SF40_DMSO | ARMS_Koster_VH2_180418–180906_ SF40_DMSO_A | ARMS_Koster_VH2_180418–180906 _IMG_ 5T_001<br>ARMS_Koster_VH2_180418–180906_IMG_ Field_001<br>*and*<br>ARMS_Koster_VH2_180418–180906_Images[_v1] | ARMS_Koster_VH2_180418–180906_ ManualObservations[_v2] |
| Describes the dates, fraction type and size, e.g. SF = sessile fraction, sieved with 40 µm mesh, DMOS = preservative used | Describes the material sample replicate, in case a fraction produces more than one sample tube | Describes the images, these being of ARMS plates of or "field" material<br>*and*<br>The name of the spreadsheet where all the images are described; Add a version number for updates | Describes the spreadsheets with manual observations of images or of the field; Add a version number for updates |
|  |  |  |  |

***Fig 2.*** *Sample terminology explaining how ARMS observatories, derived samples, images, and observation data should be named.*

### 2.5.1 Core IDs

These are the core IDs.

1. Observatory-ID, e.g. Koster. This is chosen when you join the ARMS consortium. This ID should be kept short, and using only A-Z characters.
2. ARMS-ID is the ID of an ARMS unit in the observatory, e.g. VH2. This is usually chosen

when you join the consortium. This ID should be kept short, and using only A-Z characters and numbers.

3. MasterSample-ID is the basis for all subsequent IDs; it contains the observatory and ARMS ids: ARMS_Koster_VH2.

### 2.5.2 Material sample IDs

We will use the following ID to describe and distinguish each sample (see also Fig. 2):

4. MaterialSample-ID is the ID for the material that you process and send to HCMR to be sequenced, and is to be written on the falcon tubes (Secs 4.3 and 5.1). This ID adds the following to the MasterSample-ID: the unit placement and retrieval dates, the fraction, filter size, and preservative. For example ARMS_Koster_VH2_180415-180906_SF40_DMSO.
5. For replicate material samples, you add an _A, _B, etc. to the MaterialSample-ID. For example ARMS_Koster_VH2_180415-180906_SF40_DMSO_A.

### 2.5.3 Image IDs

We will use the following ID to describe and distinguish each image of the ARMS plates or associated material/views (see also Fig. 2):

6. Image-ID is the ID for each image you take. This is explained in more detail in Sec. 4.4 and only summarised here. This ID adds the following to the MasterSample-ID
    a. *For images of ARMS plates*: the unit placement and retrieval dates, the word "IMG", the plate number, and the image number. For example ARMS_Koster_VH2_180415-180906_IMG_5B_010.
    b. *For images of anything else:* the unit placement and retrieval dates, the word "IMG", the word "Field", and the image number. For example ARMS_Koster_VH2_180415-180906_IMG_Field_003.

    The image number should always present; especially handy if there is more than one Field or plate image. What the numbers actually are, is unimportant, as long as they are different so the ID is unique for each image.

### 2.5.4 IDs for manual observations

We will use the following ID to describe and distinguish each spreadsheet you provide with your visual/manual observations (see also Fig. 2):

7. ManualObservations-ID is the ID to be used for each file of manually-made observations you create. This is also described in Sec. 4.5 and only summarised here. This ID adds the following to the MasterSample-ID: the unit placement and retrieval dates, and the word "ManualObservations"

### 2.5.5 Template spreadsheets to help create your IDs

We have created a googlesheet which contains some templates (which are described later) and a "How to make your IDs" sheet. Here you can find out how to construct your own MaterialSample-IDs, whether these samples are those in the Falcon tubes, the images you are required to also provide (Sec. 4.4), or the manual observation files you can optionally provide (Sec. 4.5). You must download the google sheet to be able to add your own data to it.

# 3. Deployment and retrieval

## 3.1. General guidelines

For deployment and retrieval, ARMS-MBON follows the standards and protocols established by the Smithsonian Institution (https://www.oceanarms.org/), with some additional guidelines.

### 3.2. 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/hyperbenthic 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.

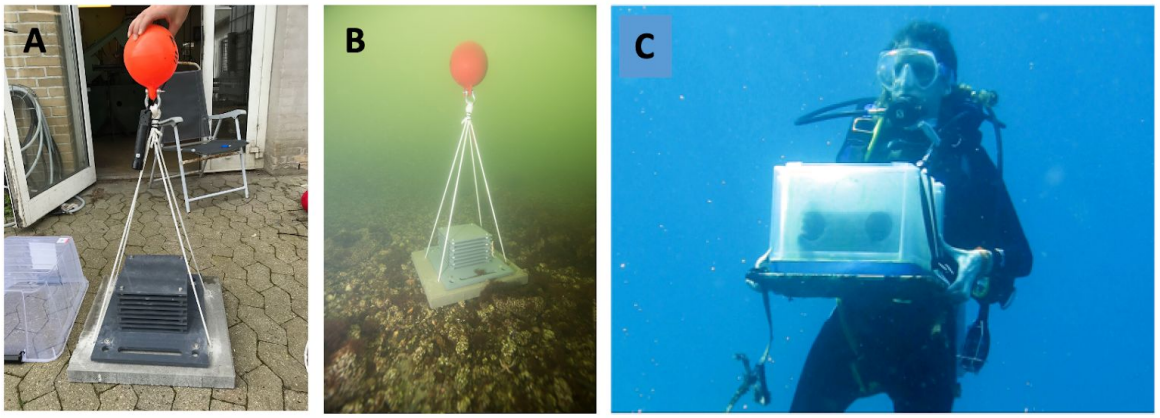



***Fig 3.*** *Photograph showing ARMS deployment mounted on a commercially-available base plate (A-B). An underwater buoy is attached to help relocate the ARMS (B).* ***C.*** *Photograph of the plastic container that is put over the ARMS on the seafloor before retrieval (C). Photograph credits A-B: ARMS-MBON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

## 4. Sample processing

For all sample processing steps, ARMS-MBON follows the standards and protocols established by the Smithsonian Institution (https://www.oceanarms.org/). *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.

### 4.1. Preservation

ARMS-MBON preserves all biological samples in DMSO (Dimethyl sulfoxide). 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://doi.org/10.1139/z91-013).

### 4.2. 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 homogenized 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 micrometer).

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

## 4.3. Material Samples

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

- 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 Falcon tubes from each material sample. You should label these as described in Secs 2.5 and 5.1, i.e. using the MaterialSample-ID. We recommend that you only ship one replicate of each fraction (material sample) and keep the remaining tubes as "backup-replicate" in long-term storage at your institution (see Sec. 6). Please remember to label these backups in the same way as the primary tubes.

Note that while it has been decided to now use DMSO, in 2018 and 2019 both DMSO and EtOH were used as a preservative and hence the preservative forms part of their MaterialSample-IDs (Sec. 2.5). To maintain consistency, we will continue with this practice. This is also useful if we ever change the preservative in the future.

Once you have shipped your material samples to HCMR, please add all necessary information to PlutoF and to the overview spreadsheet. (If you lack permission to edit the spreadsheet, send an email to the contacts listed on the ARMS webpage, where you can also find documentation for PlutoF and the spreadsheet.)

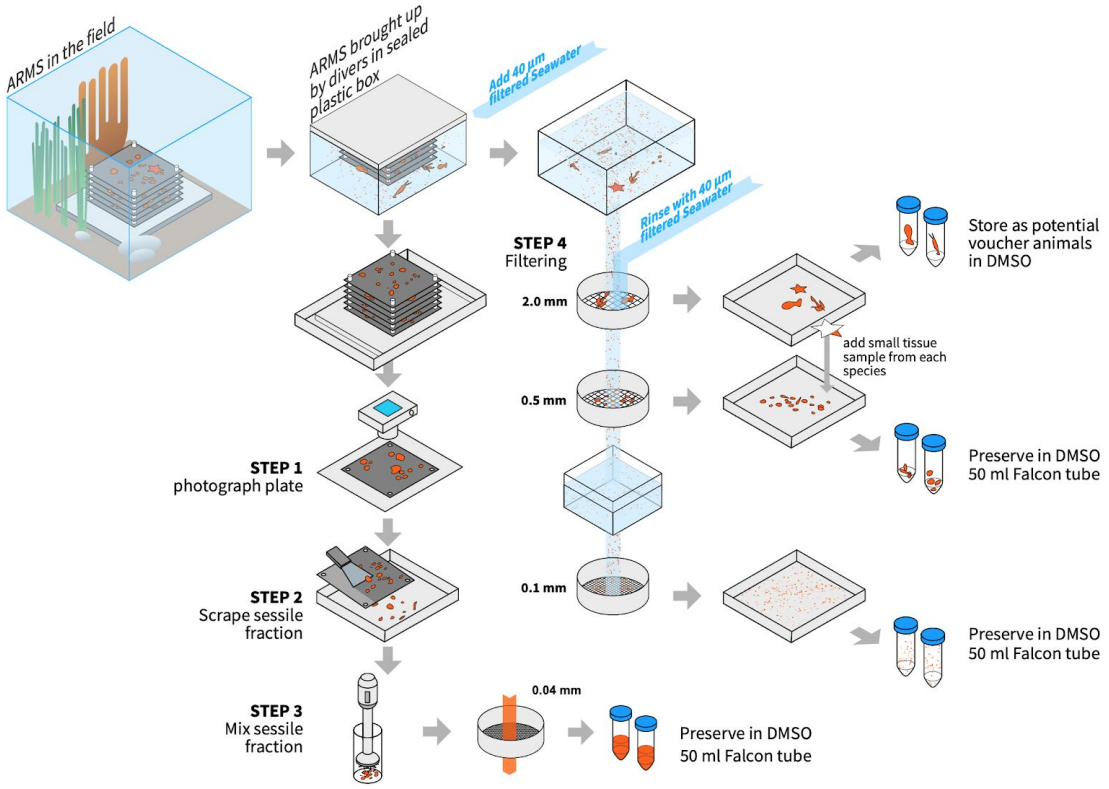



***Fig 5.*** *Schematic illustration of sample processing in ARMS-MBON. For details see the protocols established by the Smithsonian Institution (https://www.oceanarms.org/).*

## 4.4. Images

From each ARMS 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)

It is important to note the following **mandatory** elements

- the images require specific IDs, ideally these being also the filenames when you upload the images to PlutoF
- a spreadsheet providing information about the images must be provided and uploaded to PlutoF along with your images

*Image-IDs*: Please name the **image files of the entire plates** as follows:

ARMS_<Observatory-ID>_<ARMS-ID>_<DateIn-DateOut>_IMG_<Plate-ID>_<###>.tif|jpg|png
For example: ARMS_Koster_VH2_180418–180906_IMG_5B_010.jpg

The date is YYMMDD, the final number is optional and should be provided if you have more than one image. In fact, the actual number used is irrelevant, as long as it is unique. Plate-ID is to indicate the plate number being photographed: [#][T|B] (for top or bottom, counting upwards from the baseplate).

*Habitat/Specimen/Event Image-IDs*: Please label **images of habitats, specimens, sampling events** (i.e. non-plate images) as follows

ARMS_<Observatory-ID>_<ARMS-ID>_<DateIn-DateOut>_IMG_Field_<###>.tif|jpg|png
For example: ARMS_Koster_VH2_180418–180906_IMG_Field_003.jpg

The date is YYMMDD, the final number is optional and should be provided if you have more than one image. In fact, the actual number used is irrelevant, as long as it is unique.

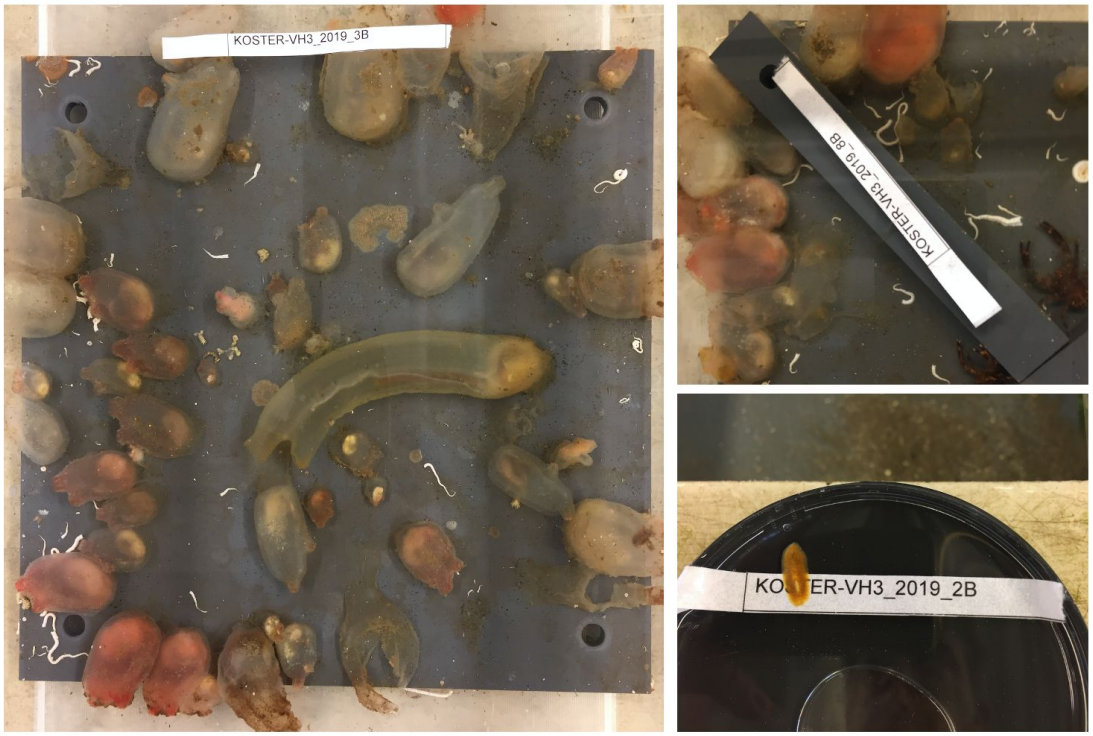



***Fig 6.*** *Examples of images of plates, close-ups, and isolated specimens, including labeling. Photograph credits: ARMS-MBON network.*

### 4.4.1 How to link your images to the image IDs

We prefer that the image file names are the image IDs. However, we appreciate that it can be a lot of work to rename all the images that you download from your camera. Therefore we offer the following workaround: provide a spreadsheet in which the images are described, so we can link the image filenames to the information that is used in constructing the images ID.

The purpose of this spreadsheet is to allow us to link every image you upload to PlutoF to its image ID as described above. It is crucial that this can be done, otherwise managing the 100s of images that ARMS will produce is an impossible task.

We have created a googlesheet which contains three templates:

1. The template for providing manual observation information (Sec. 4.5.3)
2. The template for providing image filename descriptions (sheet 2 "ARMS_Koster_VH2_180418-180909_Images")
3. A "How to make your IDs" sheet (Sec. 2.5.5)

You must download the google sheet to be able to add your own data to it. (Please note when downloading to excel, the sheet names get cut short.). Before editing, remove the explanatory rows/columns (bold font). After filling in your own version of a spreadsheet, you will need to upload it to the appropriate place in PlutoF.

The image description spreadsheet contains the following columns

1. MasterSample-ID
2. Filename of the image as you have uploaded it to PlutoF
3. Plate location
4. Image source (field or plate)
5. Comments (the only optional column)

Use NA if you have nothing to enter (no blank cells please). Please do not change the titles! Once you have created your own, upload to PlutoF with all of your images (ideally you do this just once), giving

the spreadsheet the following name

ARMS_<Observatory-ID>_<ARMS-ID>_<DateIn-DateOut>_Images.[xls,...]
For example: ARMS_Koster_VH2_180418–180906_Images

We request that you do not create multiple spreadsheets with different information: if you need to change any information or add new information, please copy the existing version, edit it, and then upload again with version number ("_v1" etc) added to the end of the filename.

## 4.5 Manual observations

Typically you will also collect manual/visual observations of species, for example during the ARMS retrieval and processing or when inspecting the images you take. These are valuable records that often complement the species observations derived from sequences. Visual observations of images of the ARMS plates or the field are also extremely useful as they provide results faster than images analysis software can. It is therefore important to link these observations to the environment where they were recorded.

These observations can be either "field" observations, which are visual observations of the ARMS plates or the surroundings, and "image" observations, which are visual inspections of the images. *Please note that visual inspection of images is not the same as visual inspection of plates.* The difference is not irrelevant: the source data in the first case are digital image files, which may later be analysed by someone else; the source data in the second case is your eyeball–brain, and no-one will be able to redo that later.

Both types of observations can be recorded in the same spreadsheet. How to properly fill the observations spreadsheet is explained in Sec. 4.5.3. The file itself should be called

ARMS_<Observatory-ID>_<ARMS-ID>_<DateIn-DateOut>_ManualObservations.[xls,...]
For example: ARMS_Koster_VH2_180418–180906_ManualObservations

We request that you do not create multiple spreadsheets with different information: if you need to change any information or add new information, please copy the existing version, edit it, and then upload again with version number ("_v1" etc) added to the end of the filename.

### 4.5.1 "Field" observations

Field observations could be the ARMS plates (e.g. a specimen from plate 4T), the ARMS site (e.g. a specimen of fish that was observed close to the ARMS during retrieval), or the ARMS motile fraction (e.g. a specimen that fell off the ARMS in the wet lab). Voucher specimens can also be recorded here.

These field observations should be written into a standardised spreadsheet so that they are interoperable with the same information provided by all other ARMS teams. The "image observations" (Sec. 4.5.2) can be written into the same spreadsheet. However, observations between ARMS units or sampling events should not be written into the same file: this is because these spreadsheets need to be uploaded to PlutoF and linked to the very specific samping event (observatory, ARMS unit, and date) that they are for.

### 4.5.2 "Image" observations

As with the field observations, the information should be written into a standardised spreadsheet. The same advice as above applies: observations between ARMS units or sampling events should not be written into the same file: this is because these spreadsheets need to be uploaded to PlutoF and linked to the very specific samping event (observatory, ARMS unit, and date) that they are for.

### 4.5.3 Templates for noting field or image observations

We have created a googlesheet which contains three templates:

1. The template for providing manual observation information (sheet 1 "ARMS_Koster_VH2_180418-180909_ManualObservations")
2. The template for providing image filename descriptions (Sec. 4.4.1)
3. A "How to make your IDs" sheet (Sec. 2.5.1)

You must download the google sheet to be able to add your own data to it. (Please note when downloading to excel, the sheet names get cut short.). Before editing, remove the explanatory rows/columns (bold font). After filling in your own version of a spreadsheet, you will need to upload it to the appropriate place in PlutoF.

The manual observation spreadsheet contains the following columns

1. MasterSample-ID
2. Image-ID (NA if not of an image)
3. Plate location (NA if not of a plate)
4. Field notes (a very short, optional, description of where the object being described came from)
5. Observation source (plate or field image or observation)
6. ScientificName
7. Taxonomic citation (please no accented fonts!)
8. Aphia-ID (i.e. from WoRMS)
9. OccurrenceStatus
10. IndividualCount
11. Comments (for longer comments)

Use NA if you have nothing to enter (no blank cells please). Please do not change the titles! Once you have created your own, upload this to PlutoF, giving the spreadsheet the following name

ARMS_<Observatory-ID>_<ARMS-ID>_<DateIn-DateOut>_ManualObservations.[xls,...]
For example: ARMS_Koster_VH2_180418–180906_ManualObservations

We request that you do not create multiple spreadsheets with different information: if you need to change any information or add new information, please copy the existing version, edit it, and then upload again with version number ("_v1" etc) added to the end of the filename.

# 5. Shipment

## 5.1. Sample labelling and address

To ship samples for centralised processing and sequencing, please prepare following these instructions.
Make sure all samples and all falcon tubes are properly labelled with the following information. Please use a printed label and NOT handwriting on the falcon tube (see Fig. 7), as that can be removed in time:

**MaterialSample-ID:**
**ARMS_Koster_VH2_180418–180906_SF40_DMSO_A**
Observatory-ID: Koster
ARMS-ID: VH2
Date in: 2018-04-18
Date out: 2018-09-06
Name: Matthias Obst
Fraction/size: Sessile fraction/40 µm (SF40)
Replicate-ID: A
Wet weight: 26 g
Preserve: DMSO

Remember to include your ABS permits in your shipment, as specified in the ARMS ABS HowTo: your IRCC code, or copies of emails, and the signed Material Transfer Agreement (MTA).

Ship the sample to the following address:

**Christina Pavloudi**
Institute of Marine Biology, Biotechnology and Aquaculture
Hellenic Centre for Marine Research
(former US Base) Gournes Pediados
71500 Heraklion Crete
Greece (Hellas)
Telephone: +30 2810 33 77 41
Mobile phone: +30 6934 17 71 86
Email: Christina Pavloudi (cpavloud@hcmr.gr)

**Important:** Please make sure you write down correctly our NAMES and TELEPHONE NUMBERS and the full SHIPPING ADDRESS, when sending your parcels. This is very important, since parcels will get lost otherwise. Please also try to remember NOT TO DECLARE ANY COMMERCIAL VALUE for the contents of the parcel and to SPECIFY, with a note on the parcel, that they are **ARMS SAMPLES** (courier companies can easier recognise the parcel and deliver it to HCMR with no delays or customs issues).

Send an email to Christina Pavloudi (cpavloud@hcmr.gr) and Matthias Obst (matthias.obst@marine.gu.se) with the shipping details and dates. When the samples arrive, Christina will send you a short confirmation email with a photocopy of the bilaterally-signed MTA.

(Note, if you only ship one replicate to HCMR, but you have kept one backup sample (Sec. 6), you can add the  A to the MaterialSampleID (“ B” is then added to your backup sample), but we will may drop the “ A” part from the final spreadsheets in which all ARMS data will be presented and published.)


***Fig 7.*** *Samples ready for shipment. Photograph credits: ARMS-MBON network.*

## 5.2. Checklist for the sample package

The following items need to be in the sample package:

1. At least three falcon tubes, i.e. at least one tube per fraction and with labels as explained above. We recommend that you keep the remaining, labelled, falcon tubes as “backup-replicates” for long-term storage in your institute.
2. Filled out, printed and signed Material Transfer Agreement (MTA)
3. ABS declaration of due diligence (see the ABS HowTo for an explanation of what this is). Please also add your ABS data to ARMS data overview spreadsheet (sheet “Permits info”).

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

### 5.4. What happens next?

Once your samples arrive at HCMR, 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 approximately every 3 months. We sequence the following genetic markers: COI, 18S rRNA (V9 region), ITS1. Once the sequences are produced, they will be uploaded to European Nucleotide Archive (ENA) (under the submission account id Webin-55576: contact Matthias Obst or Christina Pavloudi for access details) and made available through the data management platform (PlutoF) *via* their run accession numbers. You will have exclusive access to the sequences for a moratorium period of one year. Thereafter these sequences will automatically be made public. When you search for ARMS-MBON data in ENA, you may get two files for each query: (1) the original file ("submitted") and a format modified by ENA ("FTP"). We recommend you download the original file if you want to work with the sequences.

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 data management platform.

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

## 6. Biobanking

We ask all partners to keep at least one "backup-sample" replicate from each of the three fractions of an ARMS sample event, together with a copy of the legal documents (ABS PICC code or "due diligence", and the MTA) as well as a digital copy of all original images from the sample event and from the processed plates. Please mark the samples as described in Sec. 5.1 and place them in a long-term storage freezer at -20 °C or colder in your institute. Similarly, HCMR will be archiving the extracted DNA for future use and cross-validation.

## 7. Data management

The data management is described in the ARMS-MBON Data Management Plan.

## 8. Contacts

Matthias Obst, ARMS-MBON coordinator: matthias.obst@marine.gu.se
Christina Pavloudi, Sample processing and sequencing: cpavloud@hcmr.gr
Katrina Exter, Data management: katrina.exter@vliz.be