Handbook

for

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

Version

2.0

Date

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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: 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.

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

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 timeseries. 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 are separate to these ARMS (units) replicated, and are discussed in Sections 4.3 and 5.2.

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.

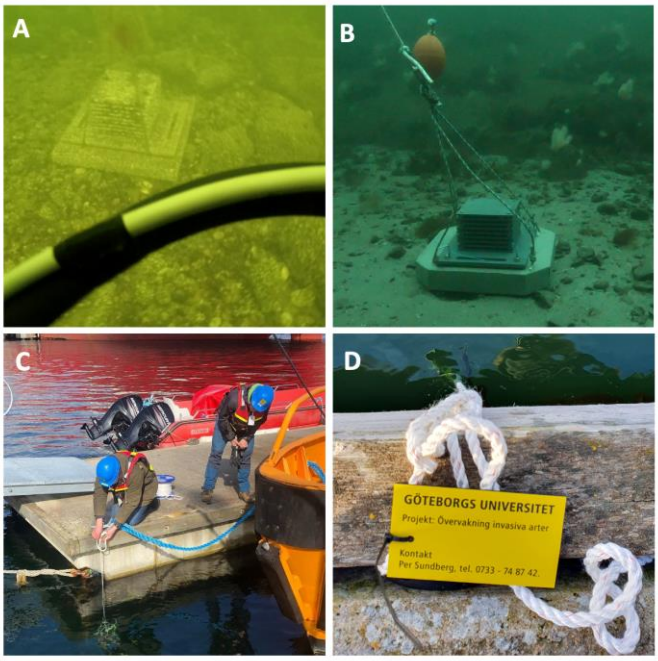


Fig 1. *Examples of ARMS deployments at LTER sites with divers (A, B), in industrial ports (C), and marinas (D). In cases where ARMS are deployed with a rope and a buoy attached, please make sure you add a weather-proof contact label with your contact address and telephone number (D). Photograph credits: 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://naturalhistory.si.edu/research/global-arms-program). 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. 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! We will use the following terms to describe and distinguish each sample (Fig. 2):

1. An observatory ID, e.g. Koster. These are chosen when you join the ARMS consortium, and are written into the ARMS data overview spreadsheet ("ARMS Observatory info" sheet)
2. The IDs for the ARMS units placed in the observatory, e.g. VH2. These are also mostly chosen when you join, and are included in the ARMS overview spreadsheet. Both of these IDs should be kept short, and please only use A-Z characters.
3. Material sample IDs are made of the observatory and unit IDs, the in and out dates, and the fraction, filter size, and preservative, e.g ARMS_Koster_VH2_180415-180906_SF40_DMSO. These are also listed on the ARMS data overview spreadsheet, in the first sheet ("ARMS metadata file"). This ID are for the material that is processed (filtered, preserved) to be sent to HCMR to be sequenced, and is to be written on the falcon tubes sent there (Secs 4.3 and 5.1).
4. For replicate material samples, this is then added to the Material Sample ID as an _A,B… at the

end of the ID.

It is important that the material samples are always referred to, in PlutoF and anywhere else, with the correct ID. We produce so much data that it will be easy for things to get lost/misplaced/confused. 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.

Note: the image below is wrong, because the preservative is not in the MaterialSampleID. Plus, you can take the text "(Fraction size)" out of that 4th column's header. And then the final column has to be "MaterialSampleID (replicate)" with the _preservative bit added before the _A.

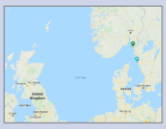
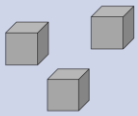
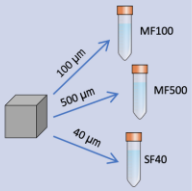
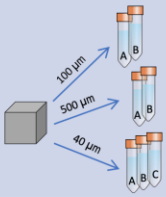
Term	Sample region (country)	Observatory-ID	ARMS-ID	Material Sample-ID (Fraction size)	Replicate-ID
Example	North Sea Skagerrak (Sweden)	Koster	VH2	Koster_VH2_180418–180906_SF40	Koster_VH2_180418–180906_SF40_A
Explanation	Describes the region of deployment and the territorial country	Describes the location of the observatory where the ARMS are placed	Assigns a unique ID to each ARMS in the observatory (i.e. spatial unique identifier)	Describes the fraction type and size, e.g. SF = sessile fraction, sieved with 40 µm mesh	Describes the material sample replicate, in case a fraction produces more than one sample tube
Illustration					

Fig 2. *Sample terminology explaining how ARMS observatories and derived samples should be named. Needs updating*

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://naturalhistory.si.edu/research/global-arms-program), 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.

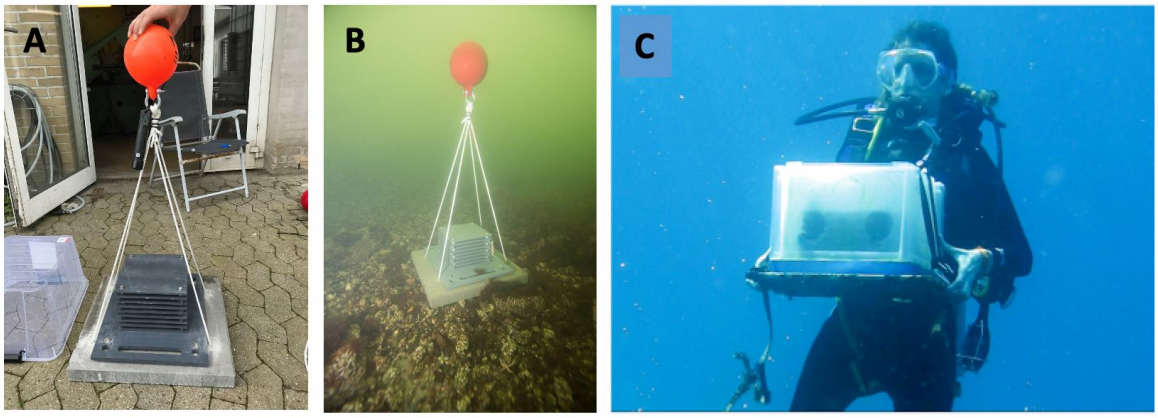


Fig 3. *Photograph showing ARMS deployment mounted on a commercially-available base plate (A-B). An underwater buoy is attached to help relocate the ARMS (B).* ***C.*** *Photograph of the plastic container that is put over the ARMS on the seafloor before retrieval (C). Photograph credits A-B: ARMS-MBON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

4. Sample processing

For all sample processing steps, ARMS-MBON follows the standards and protocols established by the Smithsonian Institution (https://naturalhistory.si.edu/research/global-arms-program). *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 (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 2.4 and 5.1, i.e. using the MaterialSampleID. 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 the past both DMSO and EtOH were used as a preservative, and this is why the preservative forms part of the MaterialSampleID (sec. 2.4). Once started, we should not stop. This is also useful if we ever change the preservative in the future.

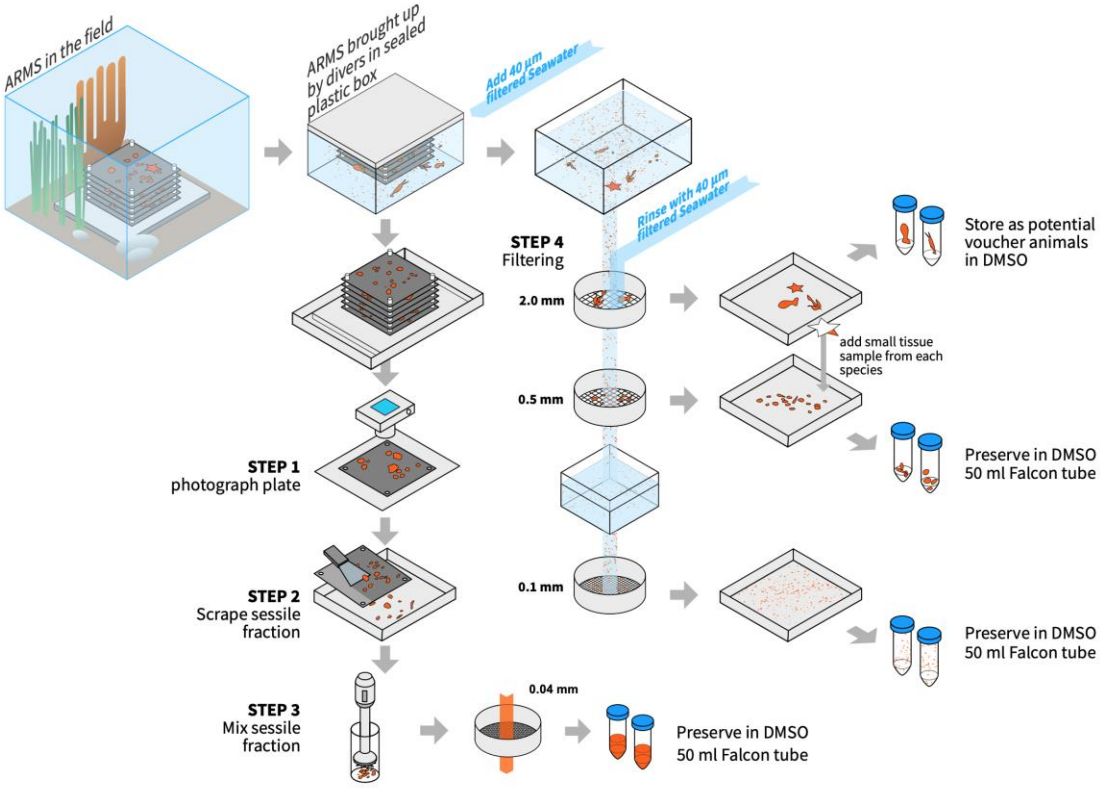


Fig 5. *Schematic illustration of sample processing in ARMS-MBON. For details see the protocols established by the Smithsonian Institution (https://naturalhistory.si.edu/research/global-arms-program).*

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)

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

ARMS_<Observatory-ID>_<ARMS-ID>_<DateIn-DateOut>_<Plate-ID>_img<###>.tif|jpg|png
For example: ARMS_Koster_VH2_180418–180906_5B_img543.jpg

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<###>.tif|jpg|png
For example: ARMS_Koster_VH2_180418–180906_img563.jpg

ARMS-ID, Observatory-ID are as in Fig. 2. Dates are given as YYMMDD. Plate-ID is to indicate the plate number being photographed: [#][T|B] (for top or bottom). For the final part of the image label, img###, please use 001, 002, etc, rather than 1,2, etc. Plate 001 is the plate closest to the baseplate, working upward, like the floors of a building. If you have both plate images and non-plate images, the non-plate ones should have their numbering starting from 001 and not from the final number of the plate images.

Now, in an ideal world the image file names will be the image ID. However, we appreciate that not everyone will want to rename their images as they upload them to PlutoF. Therefore we offer the follow workaround:

1. For plate images: write the image filenames (e.g. IMG_2342.jpg) and the plate locations (e.g. 2T) in a 2-column spreadsheet, with column titles “filename”,”plate location”
2. For non-plate images: write the image filenames (e.g. IMG_2342.jpg) and a descriptor (“habitat”, “PR photo”, “specimen”) in a 2-column spreadsheet with column titles “filename”,”description”
3. If you have both plate and non-plate images: in this case you need a 3-column spreadsheet with the column titles “filename”,”plate location”, “description”. You can leave blank entries as blank or “NA”. Do not bother to fill the “description” column for the plate images: we will not do anything with that information.
4. Save the spreadsheet with the name ARMS_<Observatory-ID>_<ARMS-ID>_<DateIn-DateOut>_Images, e.g. ARMS_Koster_VH2_180418–180906_Images[.csv or .sxlx]
5. Upload that spreadsheet to PlutoF at the same time, same place, as you do the images

Please use this filename exactly, and these column titles exactly. It is incredibly time-consuming, annoying, and frustrating to have to hunt for one spreadsheet of unknown name among 1000s of files that are downloaded from PlutoF as we prepare to publish the ARMS data. A template file is provided here.

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

4.5 Manual observations

There are two potential types of manual/visual observations that can be made: either “field” observations, which are visual observations of the ARMS plates or surroundings, and “image” observations, which are an eyeball-based inspection of the images. *Please note that eyeball inspection of images is not the same as eyeball 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.

4.5.1 “Field” 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. It is therefore important to link these observations to the environment where they were recorded. Field

observations should be written into a spreadsheet, in which Image observations can also be written, if you do both. The elements of this spreadsheet are:

1. saf
2. sdf

A template of this spreadsheet can be found here. The filename that must be given to this spreadsheet is

ARMS_<Observatory-ID>_<ARMS-ID>_<DateIn-DateOut>_ManualObservations.csv|xslx
For example: ARMS_Koster_VH2_180418–180906_ManualObservations.csv

blahblahblah

This 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). Also voucher specimens can be recorded here. We suggest the following example for the CSV file containing your observations:

MaterialSample-ID	Specimen-ID	Image-ID	...
ARMS_Koster_VH2_180418-180906	ARMS_Koster_VH2_180418-180906_img003	ARMS_Koster_VH2_180418-180906_1B_img003	...
Mandatory if the observed object is turned into a material sample (Fig. 2)	Mandatory if the observed object is on a specimen image (Sec. 4.4)	Mandatory if the observed object is on an ARMS image (Sec. 4.4)	...

...	ScientificName	Aphia-ID	TaxonRank	OccurrenceStatus	IndividualCount	Comments
...	Cryptosula pallasiana (Moll, 1803)	111343	Species	Present | Absent	4	specimen found attached to plate 6T
...	Optional (should match the Aphia-ID in the next column)	Mandatory (should match the Taxon name in the previous column)	Mandatory (write one of these: Species, Genus, Family, Order, Class)	Mandatory	Mandatory	Mandatory if the object is not associated with a MaterialSample-ID, Specimen-ID, or Image ID

Table 1. *How to record manual observations in a CSV file*

A template for this file can be found here. Note that we **strongly recommend** you: include all the mandatory columns; use these exact column titles; use NA to indicate a null value; do not use special fonts, symbols, or colours. Remember: these observations are part of the larger dataset from your observatory, and it is vital to be able to easily link them to the material sample or image data you provide. If your observation is not directly associated with a MaterialSample-ID or an Image-ID (be it the ARMS image or a Specimen image), please add a comment stating what the relationship of the object is to this particular ARMS event.

4.5.2 Image observations

From

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_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
Preserv: 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.

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 ARMS ABS HowTo for an explanation of what this is)

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.

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