Benthic monitoring methodology at Pilot 4

1. Experimental design

For the purposes of our research (monitoring of Pilot 4 – Kavo Greko transplantation sites) a BACI (Before-After Control-Impact) approach (Underwood 1994) is adopted. Within this context, the selected transplantation areas and the control sites should be sampled, ideally, several times before the coral transplantation, as well as afterwards, to allow for the detection and elimination of system variability or patterns related to other, non-tested aspects, such as seasonal, temporal, spatial or weather changes.

Benthic variables, i.e., meio- and macrofauna standing stock and diversity, are used for the assessment and monitoring of Pilot 4, as they are known to capture and reflect environmental changes and disturbance (Warwick et al. 2006, Balsamo et al. 2012). Biogeochemical variables that help characterise habitats and ecosystems and shape benthic communities are also part of the monitoring plan at Pilot 4.

2. Sampling scheme

Considering time, effort and cost aspects, the following scheme is selected to be applied for benthic sampling at Pilot 4:

Sites. Two transplantation areas are included in the BACI sampling design in Kavo Greko MPA, i.e., Vassiliki and Kryo Nero, both located at northeast of Cyprus (Figure 1). A third site, Blue lagoon, located north of the potential transplantation sites serves as the control area within the Kavo Greko MPA (Figure 1). The control site shares similarities with the transplantation sites in terms of habitat type, water depth, exposure and overall hydrography.

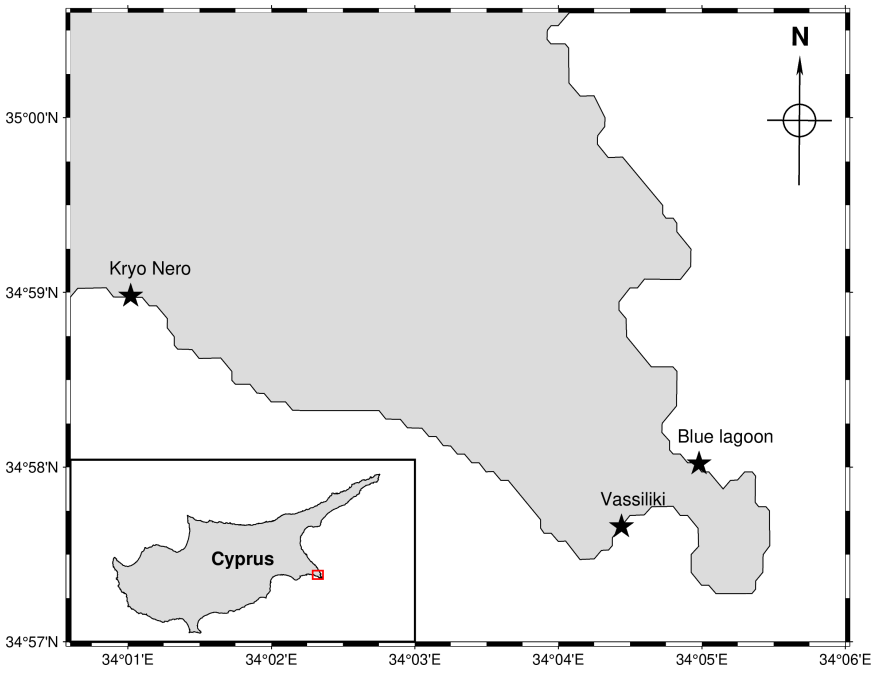

Figure 1. Transplantation sites and control area of the monitoring sampling design at Pilot 4 (Kavo Greko).

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Zonation: Sampling follows a stratified format with three (3) zones defined at increasing distance from the transplantation deployment (Figure 2):

- Zone 1, in the vicinity of the transplantation deployment (0−2 m)
- Zone 2, approximately 15 m further
- Zone 3, up to 40 m from the transplantation deployment edges.

Replication: Within each zone, four (4) replicates are randomly collected to treat environmental heterogeneity and for better representation of the zone microhabitats, if any. Samples need to be quantitative, independent and undisturbed (minimum disturbance during collection and handling).

Frequency: Both the transplantation sites and the control are sampled twice both before and after transplantation, to account for any variability related to major seasonal changes of the island (warm and cold period).

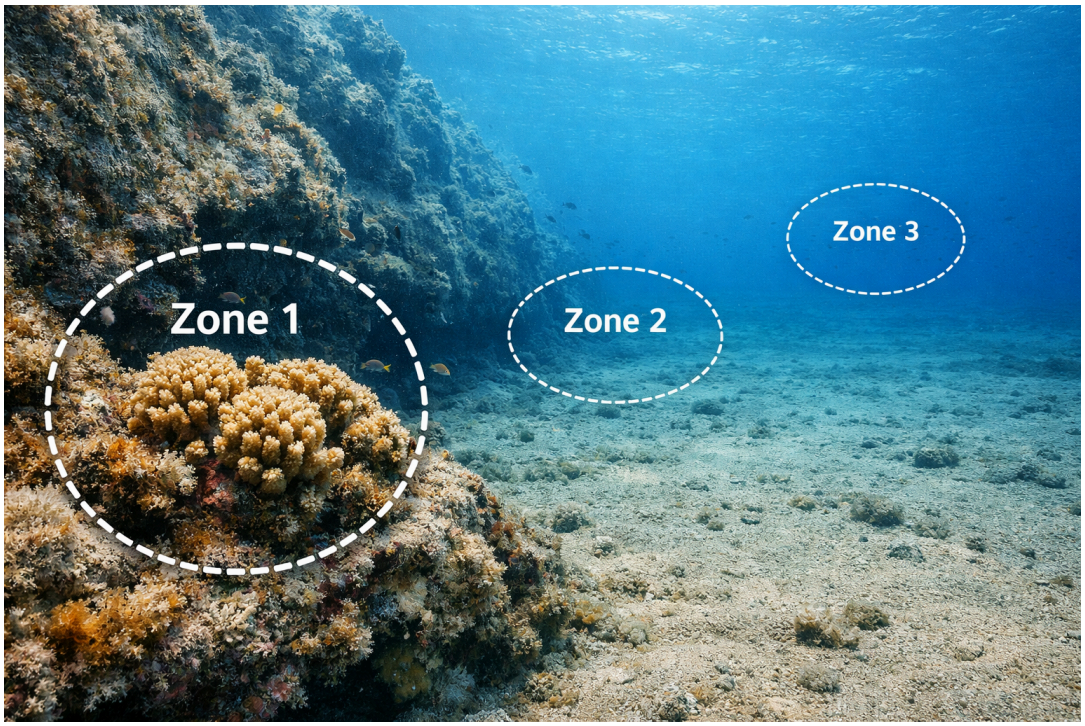

Figure 2. Conceptual illustration of the three sampling zones at the transplantation sites. Distances are schematic and not to scale. (Image generated using AI for illustrative purposes).

3. Field work

3.1. Equipment and materials

Corers	Cylindrical, transparent, perspex tubes (proposed inner diameter 4.4 cm for meiofauna and 9.4 cm for macrofauna) with smooth internal surface and beveled lower end to facilitate sediment penetration and core removal. The length should be at least 15−20 cm (Figure 3a)
Rubber stoppers	Appropriate diameter for tight-fitting to the coring tubes (Figure 3)
Core plunger	Same diameter with the corers for extruding the sediment core (Figure 3b)

Corers basket	Will ensure the upright position of the core samples and their carriage
Rubber mallet	
Slicing rings	For the vertical slicing of the sediment core
45 µm sieve	Metal stainless steel or Nytex nylon gauze for meiofauna samples
500 µm sieve	Metal stainless steel or Nytex nylon gauze for macrofauna samples[1]
Sample containers	200–1000 ml depending on sediment sample volume
Sample labels	Indicate area, sampling, station, replicate, sediment layer (date)
Syringe	~ 20 ml volume
Suction pipe	For the collection of overlying water
7% $MgCl_2$	7.5 g $MgCl_2$ $6H_2O$ dissolved in 100 ml distilled water. Used as a narcotic agent for meiofauna
10% buffered formalin (4% formaldehyde)	Filtered seawater (through a 32 µm sieve) should be used as dilutant to prevent contamination with planktonic species. The formalin should be buffered with 200 g Borax per formaldehyde liter
Washbottles	Used for distilled/filtered water and $MgCl_2$
Spatulas (plastic, metallic)	Helpful for the slicing of the sediment core
Ruler	Of at least 10 cm length for measuring sediment depth
Transparent packaging bags	Of various appropriate size for sediment storage (Figure 4a)
pH/Eh Meter	(Figure 4b)
Eh electrode probe	
Field thermometer	
Ice box	For the preservation of the samples while at field
Nylon-mesh pot scrubbers	For the set up of the ASUs (Figure 5)

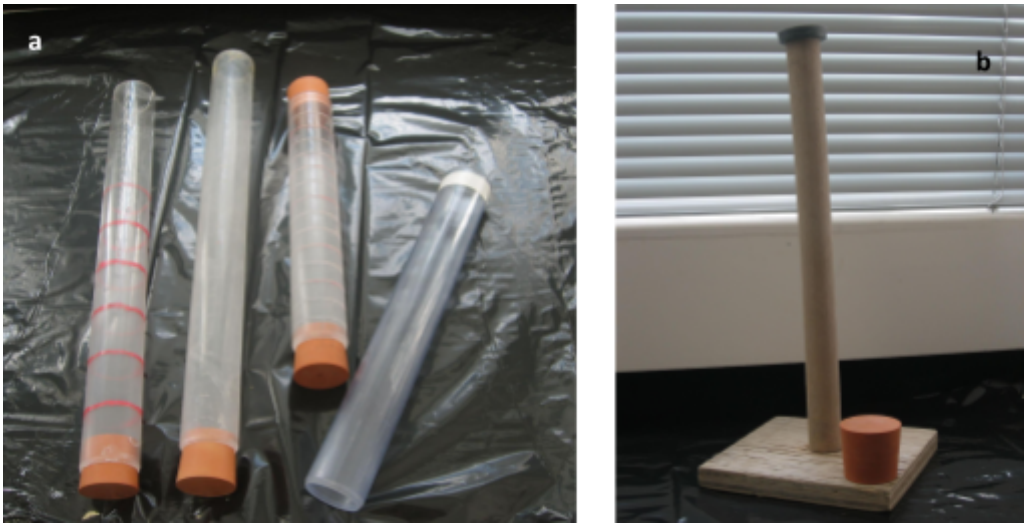

Figure 3. a) Corers with rubber stoppers and b) core plunger for shallow subtidal sampling.

[1] The sieve mesh size should much the standards for macrofauna definition (0.5 or 1 mm). For eastern Mediterranean macrobenthos experts propose the use of 0.5 mm sieves.

3.2. Sediment sampling technique

In sediments, coring is the best quantitative sampling technique for benthos, provided corers are used with care. Three general problems arise with core sampling:

- bow-wave-induced reduction in abundance
- effects on population parameters due to the underlying distribution of the fauna (patchiness)
- sample distortion due to core compaction.

These problems are minimized by selecting an appropriate core size and by sampling the sediment slowly. If at all possible, subtidal samples should be taken by SCUBA divers, because they are able to position the samplers with care and insert the corer slowly. The presence of the investigator will further yield important insights about the ecology of the site or practical aspects of the sampling.

Coring is also used for estimating biogeochemical properties of the sediment, i.e., redox potential, grain size (GSA), total and organic carbon (TC), total nitrogen (TN), chlorophyll-a and phaeopigment (chloroplastic pigments) contents.

3.2.1. Core sampling procedure

1. The corer should be inserted slowly and smoothly into the sediment in a vertical position.
2. Facilitate penetration with the use of a rubber head mallet.
3. The depth of penetration into the sediment must exceed 10 cm for meiofauna and biogeochemistry[2] and 15 cm for macrofauna.
4. When the desired depth is reached, close the corer's upper end with a stopper.
5. Remove the corer slowly. Care should be taken to retain the core while lifting the corer. If needed, remove the sediment around the corer by digging for facilitating core removal.
6. Close the lower end of the corer with a stopper and place it in the basket.

Caution must be exercised to ensure that the corer will not be turned up-side down!

3.2.2. Sample treatment

Upon retrieval, sediment cores are first visually inspected and photographed and their state is recorded. Sediment cores that are disturbed (supernatant water not clear, rough sediment surface) are strongly advised not to be further used.

Meiofauna

1. Use a separate, appropriately la beled container for each replicate sample.
2. With the use of a suction pipe or syringe transfer the overlying water of each core in the sample's container. Alternatively, the overlying water may be filtered through a 45 μm sieve.
3. Use the core plunger to extrude the core. If the surface layer is poorly consolidated and cannot be extruded without loss (e.g., soft mud), the core can be allowed to slip down the corer by loosening the top stopper.

[2] Required sample's depth is 5 cm, but due to possible sediment loss that might occur while removing the corer from the sediment, the corer should be pushed deeper in the sediment

4. Store in the container the top 5 cm of the core. Markings at appropriate intervals on the tube and the use of slicing rings of appropriate length help identify the desirable thickness of the sediment layer.
5. Add 6% $MgCl_2$ until the sample is fully covered. Stir gently and allow 10 minutes to react.
6. Fix the sample with 10% buffered formalin (for the dilution take into consideration the total volume of sediment).
7. Invert and shake the container several times to mix the fixative and sediment.

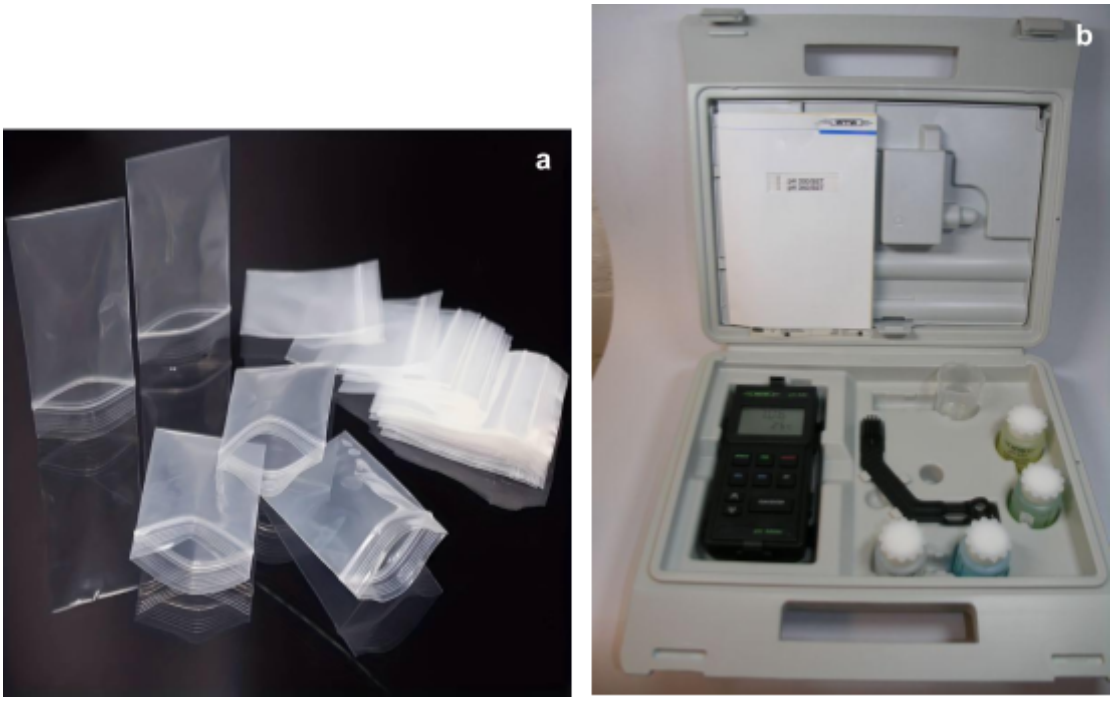

Figure 4. a) Packaging (zip) bags; b) pH/Eh Meter.

Macrofauna

1. When macrofauna samples are retrieved, the first 15 cm of each sediment core are placed in the sieve and washed gently in the sea for removing as much of the sediment component as possible. Sieving must be performed very carefully by slightly immersing the sieve into the water column in order to avoid any loss of animals.
2. The washed sediment samples are kept in 96% ethanol or preserved in 10% buffered formalin diluted in seawater.
3. To facilitate animal detection, add in the entire sample a few drops of Rose Bengal solution (24 hours is required to insure sufficient staining of organisms).
4. Shake the container for a few seconds to mix the fixative and sediment.

Biogeochemistry

1. Before the processing of the sediment cores, the redox potential (Eh) should be measured at one (1) cm intervals down to 10 cm depth. For this, the Eh probe should be gently inserted at the appropriate depth with the use of a ruler. Allow the reading to stabilize before recording.

2. The top five (5) centimetres of the sediment core[3] are subdivided in two layers: 0−2 cm and 2−5 cm. Each layer should then be split in three for three different types of analyses: TC/TN, GSA and chloroplastic pigments analysis (Figure 5).
3. Each sediment sample should be placed separately in a labeled zip bag of appropriate size and kept in an ice box until storage at –20 °C.

Figure 5. Slicing of a sediment core.

3.3. Artificial Substrate Units (ASUs) deployment

For the estimation of the invertebrate communities associated with hard substrates within Zone 1 (zone with rocks and corals), Artificial Substrate Units (ASUs) constructed from a single batch of household nylon pan scourers can be employed, as they are known to mimic coral communities (Myers & Southgate 1980, Gee & Warwick 1996, Atilla & Fleeger 2000).

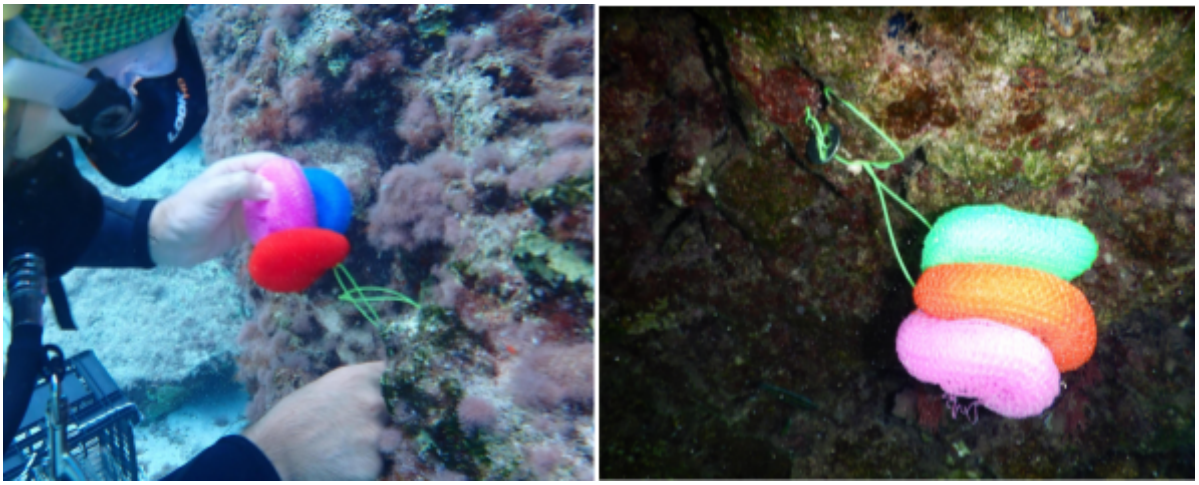

Figure 6. a) Artificial Substrate Units (ASUs) attachment at a rocky reef; b) ASUs attached on the rocky substrates (© CMMI).

To account for small-scale spatial variability, three (3) set-ups each consisting of three (3) pot scourers tightly bound together using nylon string are securely attached by SCUBA divers to crevices or small cavities on reef/rock surfaces within Zone 1 at each sampling site (Figure 6). The units are

[3] Corers, rubber stoppers and core plunger are those that will be used for meiofauna sampling

left in situ for a period of three to four months, allowing sufficient time for colonization by invertebrates. Following the overall experimental design, ASUs should be also deployed twice before and twice after coral transplantation.

During underwater retrieval, each ASU is carefully enclosed in a plastic bag to prevent loss of material during transfer (Figure 7). When on land the sample is stored in a container and preserved in buffered formalin until laboratory processing.

Figure 7. ASUs after collection, placed in zip bags for safe transport.

4. Sample analyses

4.1. Equipment and materials

Stereomicroscope	
45 µm and 0.5 mm sieves	
Ludox TM solution	Specific gravity ~1.15
Washbottles	For filtered/distilled water and Ludox solution
Containers or beakers	The volume should be approximately five times the volume of the sample
Hydrometer	A wine and beer maker's hydrometer is adequate (specific gravity 1.10−1.16 approximately)
Small containers	100 ml cup with screw cap can be used
Rose Bengal solution	1 g Rose Bengal in 1 lt of 10% formalin
10% buffered formalin (4% formaldehyde)	

Meiofauna

Meiofauna samples are first washed through a 45 µm sieve, which is the standard mesh size for coastal meiofauna. To ensure that there would not be contamination of the samples through the

freshwater supply, a plankton net of 20−32 μm mesh size is attached at the freshwater tap. Because most samples consist of a large amount of residue even after sieving, a density gradient separation method is used afterwards. A number of flotation techniques have been used but a simple and effective modification of the method proposed by de Jonge & Bouwman (1977) is used by the HCMR meiobenthic research group. This allows the processing of many samples at a time (10−20).

After extraction, each sample must be preserved by adding 10% formalin until further laboratory examination. To facilitate animal detection, a few drops of Rose Bengal solution may be added in each sample.

Organisms are sorted and identified into major taxa under a stereoscopic microscope. Depending on resources and expertise availability, other taxonomic level analysis (family, genus, species) of nematodes and copepods, the two dominant meiobenthic metazoan groups, may be considered.

Macrofauna

Standard macrobenthic techniques are used for the processing of macrofauna samples and classification of the animals (Eleftheriou and Moore, 2013). All macrofaunal animals are sorted first in basic taxonomic groups (e.g., Polychaeta, Mollusca, Crustacea, etc.) and then identified to species level, whenever possible.

Biogeochemistry

Chloroplastic pigments are extracted from the sediments with 90% acetone, and concentrations of chlorophyll-α and phaeopigments are determined according to the methods proposed by Yentsch and Menzel (1963) and Lorenzen and Jeffrey (1980) using a TURNER 112 fluorometer.

The concentration of TOC in the sediments is determined by means of a CHN Elemental Analyzer, using the protocol described in Hedges and Stern (1984).

Particle size analyses of the top 5 cm layer of the sediments can be carried out as described by Buchanan (1984) using a set of sieves for the coarser fraction (> 63 μm) and a particle size analyzer for the finer fraction (< 63 μm).

ASUs

In the laboratory, fauna is extracted by gently unravelling the pan scourers and washing their contents. The material is then sieved through a 500 μm mesh to retain macrofauna and a 45 μm mesh for meiofauna. Each benthic component is further processed as described in previous corresponding sections.

6. References

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