Mediterranean monk seal (*Monachus monachus*)

Monitoring Training Workshop

Training Manual
2.0

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Index

Introduction

The present text follows the guidelines and recommendations of the IUCN document "Huertas-Garcia, M., Muñoz-Cañas, M. (Eds.). 2023. *Mediterranean monk seal working document.* Gland, Switzerland: IUCN".

This manual includes only the basic information on how to carry out preliminary surveys to record monk seal sightings, evaluate habitat availability and use by the species, as also the photographic identification of individual animals. The contents summarize the experience acquired in time by the two "sister" non-profit organizations "Archipelagos - environment and development" (Greece) and "Archipelagos - ambiente e sviluppo, Italia" (Italy), along with the contribution for its 2.0 updated version by Middle East Technical University- Institute of Marine Sciences, METU-IMS (Turkey).

Annex I include the "Protocol for the collection and analysis of Mediterranean monk seal faeces".

The manual was produced as part of the "Med-Monk Seal Project: Enhancing knowledge and awareness on monk seal in the Mediterranean" monitoring training workshop, targeting participants from countries of the so-called Groups B and C (according to the Updated Regional Strategy for the Conservation of Monk Seal in the Mediterranean), namely Algeria, Egypt, Italy, Lebanon, Libya, Morocco, Tunisia and Syria. The project is led by the Specially Protected Areas Regional Activity Centre (UNEP-MAP-SPA/RAC) and is funded by the Monk Seal Alliance.

Chapter 1 Recording and evaluation of sightings

Accurate recording and evaluation of monk seal sightings are critical for understanding the species' distribution and conservation status. Given the challenges of monitoring endangered species, including the Mediterranean monk seal, effective data collection methods are essential for mitigating the risks of misinterpretation by influencing conservation efforts. This chapter outlines the potential biases in recording sightings, such as false negatives and false positives, and presents specific protocols for both direct and indirect interviews to enhance the reliability of gathered information.

The presence or absence of a species in areas where its conservation status is unknown or is thought to be extinct or vanished can be affected by false negative or false positive data (bias).

False absence is defined as "a non-detection that is treated mistakenly as a true absence" (Tingley & Beissinger, 2009).

Habitats formerly used by the species, where individual seals can still occur, are defined as Low Density Areas (UNEP/MAP-RAC/SPA, 2003). As reported by the GFCM (2011), "seal presence in low density areas is very cryptic and may result unrecorded in absence of scientific survey".

False negatives can lead to the underestimation of the actual situation or even to consider the species disappeared (and therefore to its classification as locally extinct).

Recording information on seal sightings in the absence of video or photographic documentation can also be affected by false positive. False positives are represented by data of sightings erroneously attributed to the subject of investigation (Tingley & Beissinger, 2009).

False positive can lead to over-estimation of the actual situation and mistakenly record signs of recolonization.

Therefore, sightings referring to monk seal presence, particularly in low density areas, require an appropriate evaluation process. It should be emphasized that the report of a monk seal encounter in general reflects more the location, time and behaviour of the witness than the presence and abundance of the seals (Panou 2009).

Specific protocol interviews should be planned carefully, applying a direct interview protocol (1.1), or an indirect interview protocol (1.2) according to the conditions, as explained below, where to carry out the survey.

1.1 Direct Interview Protocol

A specific form should be applied containing the following information:

- Information on the witness (name, address, occupation, activity during the sighting, position of the witness);
- Information on the sighting/s (date, time, duration, number and exact position of the animals, maps or drawings of the location of the encounter if possible);
- Characteristics of the seal/s (physical condition of the animal, size, colour of the fur, scars and other marks), adding picture/s or video/s if available;
- Other details (e.g., behaviour, interaction with fisheries) (Bundone et al. 2019a).

The form to record monk seal sightings, developed by members of Archipelagos - environment and development since 1985 and updated with recent information on the species, can be found in Annex II.

In order not to lose information, all the data recorded following such a procedure including those with incongruent or incomplete information, should be ranked as follows:

- Low Reliability (1): Second party reports[1] lacking clear details possibly helping in the identification of the animal;
- Medium Reliability (2): Second party reports, witness interviewed in real time or within 24 hr after the sighting, characterized by a single brief observation (<30 s at the surface), often from afar (more than 200 m).
- High Reliability (3): Sightings by one of the members of the research team or by a second party, validated through interviewing the witness in real time or within 24 hr after the sighting, characterized by close-range (<200 m), repeated and/or protracted observations (cumulative time of at least 1 min at the surface) but lacking video/photographic documentation. Second party reporters in this category may have previously encountered seals at sea and therefore be experienced in distinguishing seals from other possible encounters.
- Definite (4): Sightings backed by video/photographic documentation (Roditi-Elasar et al. 2021).

1.2 Indirect Interview Protocol

When investigating a topic such as an endangered species, some interested parties might be influenced positively (e.g., for tourism promotion purposes) or negatively (e.g., enforcement of limits to their activity such as fishers) to provide data on the subject of the interview, and thus affect the interview process with both positive and negative biases mentioned above. Questionnaires specifically designed to interview

[1] Reports not by the research team or close collaborators

such stakeholders should be elaborated in an implicit mode in order to encourage answers on the Mediterranean monk seal to avoid recording misleading or deceptive answers while avoiding to provide the interviewee with notice of the investigation´s subject in advance (Mo et al. 2011; Bundone et al. 2023).

An example of a questionnaire to artisanal fishers can be downloaded (supplementary materials S1) at:
https://www.mdpi.com/1424-2818/15/6/740

It is important to highlight that, in the absence of systematic monitoring activities, seal sightings only provide general information on the species´ occasional presence. This type of data does not provide qualitative (e.g., habitat use, home range), nor quantitative information (e.g., number of animals frequenting the area) unless it is accompanied by data obtained through other monitoring methodologies.

Chapter 2 Habitat surveys

In former times, the Mediterranean monk seal used beaches and marine caves to haul out, rest and give birth. Nowadays, the species has retreated from coastlines highly used by man, and marine caves represent their main terrestrial habitat. An ideal breeding cave should have one or more entrance/s, preferably under water (syphon), one or more internal sandy or pebble beach/es or rocky platform/s above the sea level, as also an internal basin not directly exposed to the open sea currents and waves. Resting caves may display less characteristics as the above one but have at least one or more beaches or platforms above sea level in their interior (IUCN/UNEP 1988; Gücü et al. 2004).

Habitat surveys to identify available and suitable coastal locations (i.e., marine caves) for the species should be preceded at least by i) a thorough investigation of historical information on the species' occurrence and habitat known to have been used by seals; and ii) recompiling of all available data on the geomorphology of the coast in order to:

- Identify areas suitable for the species, at least in the past they were in use by the seals.
- Identify sectors of the coast where marine caves are present/concentrated (cliffs).
- Exclude extended sandy beaches with no rocky parts at all; thus, certainly not containing suitable caves.

Note: Evaluation of sightings and e-DNA analysis may represent additional surveys to be carried out in conjunction with the habitat survey.

Once the stretch of coast to be investigated has been identified, it is necessary to appropriately plan the monitoring activity to be carried out.

The selected coastline should be surveyed with the aid of a speed boat (preferably) or kayak (eventually). Each entrance/hole should be checked by snorkeling to identify available and suitable habitats. The suitable caves detected should be mapped with the support of underwater writing devices (underwater dive slate or waterproof notebooks) together with a pencil and eraser, a diving compass, and a GPS data logger, for recording the following data:

- Orientation of the entrance
- Orientation, length and width of the channel and internal basin
- Orientation, length and width of the beach/es or platform/s
- Slope of the beach/es or platform/s
- Ceiling height.

The different measurements can be estimated by eye or, more accurately, with the support of a tape measure or laser meter.

All the information related to the caves and their location should be stored for future reference.

Note: Unknown caves with only underwater entrances/accesses should be surveyed only by an expert speleo-diving team.

Once suitable caves have been identified, it is possible to monitor them with the use of camera traps for verifying their actual use by the monk seals (presence/absence).

The selection of caves for monitoring depends on a combination of various factors, including the duration of the planned monitoring, season, accessibility, cave type (resting or breeding), cave characteristics, and the number of available camera traps. It is advisable to monitor all suitable caves year-round, particularly if the goal is to investigate the use or non-use of caves by seals for the first time or to verify of cave use by seal that are widely known to have been used by seals in the past. If monitoring all the suitable caves is not feasible, priority should be given to the most critical ones, such as breeding caves.

Chapter 3 Photo-identification

3.1 Morphology

Mediterranean monk seals can be individually identified from the coloration patterns of the fur and from the presence of permanent scars on their body (Samaranch & Gonzalez 2000; Forcada & Aguilar 2000).

Morphologically, the following classes can be distinguished:

1) Pup (P): with black fur (called “lanugo”) and a ventral whitish/yellowish patch. The ventral patch is like a fingerprint: its shape is characteristic for every single individual. The patches’ general shape allows to distinguish females from males. In females, the caudal part of the patch ends more or less as a straight line close to the tail. In males, it reaches or almost reaches the umbilical slit, leaving the penis opening outside the patch.

2) Juvenile (J): after the moult of the pup’s fur with about 2-3 months of age, the juvenile´s fur is light greyish on the back and continuously whitish on the belly. The absence of scars makes it difficult to identify the animal and determine the sex in this stage. However, the remains of the ventral patch are mostly still visible on the sides of the animals. Determination of the sex is only possible if the ventral part can be clearly seen, unless the animal has been followed since the previous stage (P). In the initial phase, the animal can be classified as Youngster (Y), with a rounded appearance, often still suckling, which includes recently moulted pups being weaned.

3) Subadult (SA): animals should be classified in this category after the previous stage (J) and until they reach sexual maturity. The appearance is grey on the back (light to dark) and whitish ventrally. Seals in this stage begin to show scars mainly caused by the interaction between each other. It is still difficult to distinguish males from females unless the ventral part is observed (as above). Animals should be moved to the following categories (AF and AM) when evidence of sexual maturity is documented (i.e., mating, giving birth, lactating for females, or adult male appearance).

4) Adult Female (AF): The seal´s appearance, as in the previous category, is grey (dark) on the back and whitish on the belly. The animals bear multiple scars, which tend to accumulate, with age, on their backs over time due to mating interactions.

5) Adult Male (AM): the final morphological stage of adult males is characterized by black fur all over the body and by the complete re-appearance of the ventral white patch. Mature males display numerous scratches across their bodies resulting from intense social interactions, particularly concentrated along the throat and hind flippers.

(Badosa et al. 1997; Badosa & Grau 1998; Bundone et al. 2019b; Bundone & Panou 2022, 2023; Cantos et al. 1997; Cedenilla et al. 2017; Gazo et al. 2000; González et al. 1997; Grau et al. 1994; Koemtzopoulos et al. 2022; Muñoz Cañas et al. 2009).

Note: during the pre-moult phase, the animals appear uniformly brown, or with brown fur patches during the moult (Badosa et al. 2006).

3.2 Data recording

Pictures or videos of monk seals can be collected through:

1) Citizen Science
2) Opportunistic surveys
3) Systematic surveys

1) Citizen science

Citizen science should be linked to the direct interview protocol (see related paragraph before). In general, but not always, they are characterized by low quality and provide less information than the one obtained through specific surveys (see below).

2) Opportunistic surveys

Specific surveys designed and organized by experienced researchers using reflex cameras (SLR). Pictures can be recorded from the land or at sea at specific locations known to be frequented by seals. They can coincide with other surveying activities (e.g., habitat surveying and monitoring). They are characterized by pictures of higher quality. When photographing at sea, seal identification can be challenging as most of the body will be underwater. To obtain valuable data for each animal it should be essential to collect dorsal and lateral (both sides) view pictures. Whenever possible, pictures of the ventral part should be also taken if it happens that the seal exposes it.

3) Systematic surveys

Systematic surveys can be carried out with the use of camera traps installed in marine caves. This system can be used to i) verify the presence/absence of seals in caves assessed as suitable for the species (see habitat survey chapter above); or ii) monitor caves already known to be used by seals.

There are various models of camera traps available on the market and their choice depends on several factors (e.g., previous knowledge of the cave´s use by seals, geological characteristics of the cave and available funds).

Below, and without going into details, which would go beyond the scope of this guide, some general guidelines on the use of infrared camera traps are given.

The choice of a camera trap model should follow the general rule: minimizing disturbance and costs while maximizing coverage and results.

A camera trap features an external protective shell with locking clips and an internal protective O-ring. On the frontal part, the camera features flash emitters, a PIR[2] sensor and a camera lens. Some models also have the movement test sensor light.

By opening the locking clip, the internal part of the camera which houses the battery compartments, the memory card slot, the power switch, the menu buttons, and the programming/viewing screen can be accessed.

The various camera models available have battery compartments that can house a varying number of batteries in general from 8 to 12. An external battery can be added to the system which can alternatively also be powered by solar panels. However, this choice, while expanding the energy supply over time, requires a more invasive intervention and considerable additional costs. In general, depending on how they are programmed (i.e., videos, photos, and their amount to be recorded over time), camera traps can work from 3 up to 6 months.

The choice of a lens for each camera trap model depends and varies according to the morphology of the place where they need to be installed (wide angle/narrow angle). It is essential to choose the appropriate location to place the camera in order to cover most of the dry area where seals may rest. Adequate anchoring systems for housing the cameras are necessary and need to be evaluated based on the different morphological characteristics of the cave, but always should guarantee: relatively easy accessibility for the researcher team to reach and replace the camera, wide coverage, protection of the camera, manoeuvrability of the camera once it is installed (view orientation), and protection so that the system is not stolen or damaged.

Batteries and SD card models to use may vary depending on the camera model. It is essential to carefully read the camera manual, or the information provided by the seller, and purchase accordingly. In general, the best choice is non-rechargeable lithium, low leakage batteries. The choice of SD card capacity (e.g., 36 GB, 64GB, 128 GB) depends on the camera trap model to be used, every brand and model has

[2] PIR (passive infrared Sensor) is an electronic device that measures infrared (IR) light radiating from objects in its field of view. PIR sensors are most commonly used in PIR-based motion detectors.

certain limitation in SD card capacity and also what will be recorded (e.g., videos are heavier files than pictures, quantities of video/picture recorded over time). Using the maximum capacity supported by the camera is advisable.

Programming. Camera models can work i) in time-lapse (programmed to shoot at specific intervals of time), ii) in triggering mode (shooting after movement), or iii) in a combination of these two. For the monitoring of the monk seal in caves, the programming mode to be chosen can vary according to the situation and the specific needs.

Camera trap set-up, deployment and recovery: Most camera traps feature photograph, video, and hybrid modes. The hybrid mode captures both still photos and videos with each trigger, making it particularly useful for studying behavioural data, while photos are preferable for individual identification. To conduct photo-identification analyses, it's essential to use the highest resolution setting for camera photos. Conversely, for presence/absence data collection, a lower resolution can be sufficient. When selecting video capture mode, it's important to adjust the recording duration based on the length of deployment, battery life, and memory card capacity. Accurate date and time stamps are crucial for the data stored on memory cards. Therefore, the built-in clock should be carefully set, and the timestamp mode must be activated. Some camera traps include built-in temperature and moon phase stamps, which can provide additional insights.

Most commercial camera traps can be programmed to take photos or record video clips at your selected time intervals, reducing the risk of the memory card becoming overloaded with redundant images and extending battery life. To minimize disturbance, the interval between consecutive activations should be set to 20 minutes or longer (Gücü, 2009). For sensor settings, it’s recommended to use the auto option or, if unavailable. If other wildlife, such as bats or rats, are present or possible to be present in the cave, the sensor sensitivity should be lowered to prevent unnecessary activations from this wildlife.

The placement of camera traps is carefully planned to capture optimal footage in areas where animals frequently haul out within the cave. The number of traps deployed depends on the cave’s size and morphology and location that is used by seal(s).

As ideal practice, the camera-trap in the cave should be replaced with another unit, as the former one requires maintenance due to harsh conditions in the cave. However, if the replacement camera is not available, the recovery can be only replacement of the SD card and the camera-trap remains in the cave for a subsequent survey.

In the latter situation it is better whenever possible to avoid the opening of the camera and substitution of the SD card while inside the cave.

3.3 Organization of the data

Regardless of the methodologies used to collect monk seal pictures (i.e., citizen science, opportunistic surveys, systematic surveys), all raw data collected must be systematically sorted and organized.

Pictures should be saved in folders and sub-folders according to how the surveys are conducted and the data collected. All the data must be accompanied by spreadsheets reporting the main information. It is essential to thoroughly plan the survey having a clear idea well in advance about the survey´s aims (i.e., what exactly one needs to achieve as a result of the investigation); thus, designing the spreadsheet accordingly.

Spreadsheets should be organized according to the type of survey (e.g., citizen science, opportunistic, systematic), with reference to the survey and how the data is stored (e.g., folder, sub-folder, pictures code), and report data accordingly (e.g., date, time, presence/absence of seals, number of animals present, identified animals). For reasons of comparability, the same type of spreadsheet should be used, at least for the adjacent coastlines or for a follow-up survey of the same coast.

The pictures collected should allow the design of a catalogue of individuals. Photo-identification of animals is based on photographic capture/recapture. A capture represents the moment when an individual is first identified. To avoid overcounting, a capture should include pictures of at least the back and both sides of the seal, as a minimum requirement to uniquely identify the animal.

A photo-identification catalogue should consist of cards representing each animal, showcasing the most distinctive images of the individual. These cards should also provide details on capture and recaptures, locations, the date of first sight and classification of animal (as pup, juvenile, subadult etc.) at first sight. Additionally, whenever possible, they should also provide information regarding the mother-pup relationship or distinctive behaviour or characteristic that this animal shows.

This is an ideal (perfect) situation. However, unfortunately it is not always that much straightforward, especially for a researcher or surveyor who is just beginning to explore a new habitat (that is the case for group B and C countries) and encounter a seal. Moreover, even one or series of single events without recapturing the animal later has relevance. Therefore, every single data should be stored. In such a case ID cards, as initial simplify version of the catalogue, should be prepared, according to the picture collected in conditions with limited data/information availability even if not realizing a complete capture. See the example developed by the Middle East Technical University-Institute of Marine Science in Annex III.

Chapter 4 Strandings

If any entangled, stranded, sick, injured, or deceased animal is encountered, first of all it should be reported to relevant local or national authorities, as well as available information/rescue/stranding networks. Maintaining a safe distance to minimize stress for the animal and avoid any potential harm is important.

To maintain the scope of this guideline, the focus of this section is on the data collection aspect of the topic. The information provided below is intended to systematically and standardize the collection and documentation of any occurrences of stranded monk seals that are encountered and to mitigate information loss.

The collection of stranding data is considered important for several reasons. Firstly, it offers valuable insights into the biology, ecology, behaviour, and overall health of marine populations. Secondly, it aids in identifying the causes of mortality and the threats facing these populations. Additionally, it informs the formulation of targeted conservation strategies and protective measures. Lastly, it serves as an indicator of shifts in habitat quality or changes in the ecosystem that may be affecting the animals.

A data recording form should be created that includes fields for the following information: date and time of the stranding, location (for exact location via GPS coordinates), sex, size (, stage (pup, juvenile, subadult, adult) of the animal and its condition (alive, dead, decaying - if so decomposition stage of the animal), any visible injuries or abnormalities, environmental factors (weather, tide conditions, any unusual phenomenon observed in the location).

Digital cameras or smartphones should be utilized to document the condition and appearance of stranded animal. If it is safe to do so (for example if the animals found death) measurements of the animal should be taken using measuring tape, and GPS devices should be employed to accurately record the locations of stranding. Tissue, fur, and any other relevant samples that are available should be collected (if permitted) and stored under appropriate conditions until analyses are performed.

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ANNEX I

PROTOCOL FOR THE COLLECTION AND ANALYSIS OF MEDITERRANEAN MONK SEAL FAECES

1. Introduction

The Mediterranean monk seal is one of the most endangered top predators in the Mediterranean Sea. Its population and distribution are impacted by various threats, and recently, a new potential threat has been identified: marine debris.

Interactions between marine debris and pinnipeds have been reported in different areas. The Mediterranean monk seal is not an exception. Individuals from the Cabo Blanco Peninsula (Mauritania) have been reported entangled in discarded nets (Marchessaux 1987).

A previous study found microplastics presence in seal faeces dating back to 1999 (Hernandez-Milian et al. 2018). Greater research efforts are needed to understand how these particles, move through marine food webs and their impact on population health.

Due to the behavior of the species and the orography of the area, Mediterranean monk seals typically rest in hard-to-access caves with internal beaches. The approach to these caves is usually by small boat from the sea and entering swimming, which limits the available space for sampling. The protocol designed for this purpose has been developed over the last six years and follows the guidelines proposed by Lusher & Hernandez-Milian (2018). It aims to use the minimal materials to facilitate not only the transport of equipment but also the processing and analysis of the samples.

The collection and analysis of faeces require two different set of guidelines; therefore, this protocol is divided into “field sampling” (the collection of faeces in the field and their transport to the laboratory) and “laboratory analysis” (the processing and analysis of the samples in the laboratory).

2. Material

2.1 Field sampling

The material needed for the collection of faeces is:

- Plastic zipped bags (20x20cm, 1 for each sample)
- Aluminum foil (30x30 for each sample)
- Box or large bag to store samples
- Notebook
- Pencils (do not use pens because pen inks will smudge our notes)
- Waterproof paper
- Camera (with full battery and empty card)

2.2 Laboratory analysis

The material need for the analysis at the laboratory is:

- Set of sieves with different mesh size. Three desirable sieves should be used: 1000um, 300um and 100um mesh size.
- A hose attached to the tap to direct the water direct to the sieves.
- A tap filter or a handmade filter (a tight blended at least twice and attached to the tap).
- Filter paper (e.g. glass microfibre filters), dry lab paper or filtered water in at least two petri dishes. They will work as blanks for airborne contamination.
- Laboratory gloves
- Cotton and white laboratory clothes.
- Flasks and beakers
- Glass jars.

- Vials
- Eppendorfs
- Aluminum foil lids.
- Ethanol absolute (99%) and 70%.
- Reagents to digest organic material: Potassium hydroxide (KOH, 10%) or hydrogen peroxide (H2O2, 30-35%).
- Reagents to separate microplastics by density: Sodium chloride (NaCl) and Sodium iodide (NaI).
- A vacuum filtering flask assembly for 47 mm filters with funnel, filter folder (Buchner filter).
- A vacuum pump.
- Non-plastic dissection tools (including scissors, forceps and scalpel).
- A stereomicroscope with a camera.
- An incubator/oven and shaker.
- Paper labels.

2.3 Labels

All samples should be labelled with type of sample (MP, microplastics; D, diet; G, genetics; P. parasites), place (B1, B2, B3), site (U1, U2, U3) and date (dd/mm/yyyy). This code should be writing down in the field notebook with the coordinates of the place and date.

Codes should be in this format: MP.B1.U1_10/05/2018 (*Microplastic sample from B1U1 collected on the 10 May 2018*).

3. Protocol

3.1 Pre-field sampling

Information related to the area where the samples are collected should be recorded using the provided recording form to the best of one's ability. The sea state, wind, and visibility conditions are detailed in the Forms and Scales section.

If seals are present in the area, notes should be taken recording size, sex, behavior of the animals if possible. Photos should be taken if there is marine debris in the area or if anything unusual is observed. Additionally, a photograph of each sample should be taken before the collection.

3.2 Filed Sampling

Laboratory gloves should be worn when collecting samples; however, the person collecting the sample should avoid touching the sample with the gloves. If this is not possible, it should be noted.

Faeces should be separated by at least 2 meters from each other; if two faecal samples are closer than 2 m, this should be noted, even if the person collecting the samples is confident that they come from different animals.

Each sample will be collected with aluminum foil, avoiding any direct contact with plastics and placed in a plastic bag.

Each sample should be labeled both on the outside and, if possible, on the inside. The label should include the number of cave/sample area, and the faeces number if more than one is collected from the same cave. Additionally, the date of collection or year should be included. For example, faeces 1 in cave 1 could be labelled as: MS-S1-C1-19

3.3 Post-field sampling

Samples should be transported from the field to the lab in a closed container to minimize airborne or external contamination. It is recommended that the container be kept in the coolest place possible to avoid exposure to high temperatures.

If analyses are conducted within the next few days, samples should be stored in a refrigerator. If the analysis of the faeces is delayed, the samples should be frozen.

3.4 Laboratory analysis

3.4.1 General information

Microplastics are ubiquitous materials that can be found everywhere. To reduce airborne contamination, researchers should work in a small lab that has been closed off since the day before the analysis. Researchers should ensure that no air currents are present during the processing and analysis of samples, as air conditioning or heating can create currents that disperse microplastics throughout the room when carried out microplastic analysis. Only one or two people should be working in the lab, minimizing movement as much as possible. This will help prevent microplastics that settled overnight from being disturbed and re-entering the air.

Researchers should wear white cotton clothing, as any white fibers (which are the least common colour fibers found in studies) will be removed from the sample if they are present. Two or three filter papers will be placed in Petri dishes around the working area, ensuring they not get wet. If filter papers are not available, dry lab paper or water can be used; however, extra care should be taken with dry lab paper, as strong movements by the researcher may cause fibers fly out from the filter. A tap-filter should be attached to the tap to prevent external contamination from water. If a tap filter is not available, a handmade filter made from a pantyhose can be used; it should be blended at least twice and attached to the hose.

All material to be used should be rinsed with filtered water before the samples are poured into the sieves. The working area should also be rinsed with filtered water and dried with paper prior to use, ideally three times.

3.4.2 Subsamplings

There are many studies that can use subsamples of faeces. Here, we present three types of subsamples for specific studies conducted by Archipelagos - environment and development and Archipelagos - ambiente e sviluppo, Italia. The subsamples should be taken from the faeces prior to processing and should be taken from the internal area of the samples. One subssample will be collected in absolute ethanol for genetic analysis to identify the prey items using the metabarcoding technique. A second subsample will be collected for plastic additive analysis (phthalates, porphyrins) and stored frozen (at -20ºC). A third subsample will also be collected in absolute ethanol for parasite analysis. These samples will be taken if such studies are planned.

3.4.3 Faeces processing

If the faeces are too dry, it will be difficult to analyze them. In this case, it is advisable to place the faeces in a jar with filtered water for 12-24-48 hours to dissolve them and facilitate the further processing.

Pour the faeces into the set of sieves (the large mesh size on top and the smallest on the bottom) and rinse the sample under running tap water (with the filter attached). Use your fingers or any non-plastic tools to help dissolve the faeces. Large items will be removed from the first sieve (1000um) and transferred to jars or vials containing 70% ethanol. These items should be cleaned before being transferred to ethanol. It should be noted that prey remains, parasites, and anthropogenic debris will be separated into different vials at this point. Once the first sieve is free of remains and clean, check the second sieve (300um or 250um). Follow the same procedure, and prey remains, parasites and anthropogenic debris can be included in the previously used vials. After ensuring the second sieve is free of remains and clean, check the last sieve (100um). Since all the material in this sieve will be transferred for microplastic analysis, any prey remains or parasites found should be removed and placed in the appropriate vials. Concentrate the material in the sieve toward the edge to facilitate pouring it into a glass jar. Position the sieve vertically on top of the jar and use a scalpel or spoon to transfer the sample. For material that is difficult to remove from the sieve, tap water can be gently used with a hose; however, use as little water as possible. Cover the jar with aluminum foil to prevent airborne contamination.

3.4.4 Diet analysis

3.4.4.1 Sample preparation

The remains stored in 70% ethanol will be dry after at least 2 hours. They can be stored in ethanol for up to 3 months; after that period, bones and, especially, otoliths may be affected by the chemical. This process helps reduce mold production and hygiene the sample.

Hard structures will be dried in paper or polystyrene trays. To accelerate the drying process, paper trays can be placed in an oven at 60ºC for 2-4 hours, checking regularly to ensure the samples do not over-dry. Polystyrene trays cannot be placed in the oven, as they may melt between 60 and 80ºC. Once dry, the samples

will be stored in vials, jars or plastic bags. If the sample is large, otoliths should be stored separately from the bones.

Beaks, crustaceans, and parasites should be stored in 70% ethanol in separated vials. *Posidonia* should be stored in vials or plastic bags.

3.4.4.2 Identification

Samples will be poured in Petri dishes. The following steps are recommended:

- Otoliths will be separated into left and right. They should be identified to the lowest possible taxonomic level. When left and right otoliths from the same species are similar in shape and size, they can be considered from the same individual. The number of individuals in the sample will be the maximum number of left or right otoliths of each species. Sometimes, otoliths from one side are chosen due to the larger quantity, but there may be larger or smaller otoliths from the other size that should also be considered. Example:
    - We have 7 left otoliths of labrids and 6 right otoliths of labrids.
    - Most of them are around 3-4mm in length (otolith length, OL), except for one right otolith that measures 5mm.
    - Therefore, I have 8 labrids: 7 from the left otoliths and 1 from the larger right otolith.

  Otoliths will be identified using available guides, atlases, and publications, or using reference collections (Harkönen, 1986; Lombarte et al. 2006; Leopold et al. 2001; Tuset et al, 2008).
- Bones. Most of the researchers primarily use otoliths due to the lack of information for fish identification using bones. However, it has been demonstrated within the last two decades that at least 20% of the fish can be identified using only bones. Additionally, the degree of erosion of some otoliths may only allow researchers to identify fish at higher taxonomic levels, while bones can aid in species identification. The procedure for examining the bones is the following:
    - Separate the different bones and count the left and right ones of each species.
    - If the maximum number of left and right bones for each species is less than or equal to the number identified by otoliths, no further work is needed.
    - If the maximum number of left and right bones for each species is greater than the number of individuals identified through otoliths, then the bones should be measured and included in the analysis.
    - If otoliths were identified at a higher taxonomic level but bones confirm the species, either the otoliths or the bones can be used for further analysis.
- Cephalopods have two beaks in their mouths: the upper beak and the lower beak. The lower beak is usually the easier one to identify. The number of individuals will be determined by the maximum number of either upper or lower beaks of each species. The same situation described for otoliths may occur with beaks. Beaks can be identified using available guides, atlases, and publications, or by using reference collections (Clarke, 1986; Pedâ et al., 2022).
- Crustaceans. Only parts of crustaceans have regularly been found. It is unknown whether marine mammals consume these small animals or if they come as secondary prey (prey from prey that has been ingested). However, lobsters have been reported as prey for the Mediterranean monk seal in Atlantic waters, and they should be taken into consideration. Additionally, grey seals have been observed feeding on Norway lobsters. Therefore, these should be considered for identification purposes.
- Other dietary items. *Posidonia oceanica* has been recorded in the feces of the Mediterranean monk seal. It is unclear whether it is ingested intentionally or not, but it is well known that carnivores can use plants to aid in the digestion process. Another common item found is the mandibles of polychaetes; in these cases, they should be noted and identified, if possible, but not included in the diet analysis.
- Parasites. This is a valuable resource for the parasitology community. If your lab does not work with these species, it is recommended to contact other research groups for species identification.

3.4.4.3 Size and biomass estimation

The importance of dietary analysis is to estimate the biomass of prey consumed by a predator. This information will enable an estimation of the annual food consumption of the population and its interaction with fisheries and aquaculture.

The measurement of otoliths follows Harkönen (1986) guidelines, measuring otolith length and width. Several papers also include back-calculation regressions for estimating the size and/or weight (biomass) of prey. Based on our experience, the back-calculation regressions for biomass using otoliths are not as reliable as those for size. Therefore, it is recommended to use length-weight regressions available in the literature (see Fishbase - www.fishbase.se).

The measurement of beaks is typically conducted on lower beaks, as most references provide regressions for these. Measurements will be taken in the rostral area (rostral length for most squid species) or the hood area (hood length for octopuses and sepiolids).

The rest of the remains will be measured based on the available references and the remains found in the faeces.

3.4.4.4 Estimation of the dietary importance

Four main standard indices have been used widely to describe the diet of both terrestrial and marine predators.

- Frequency of occurrence, %F:

  $$\%F = (Fi / Ft) * 100$$

  Where Fi is the number of digestive tract/scats containing the prey type "i", and Ft is the total number of stomachs/scats containing food

- Percentage by number, %N:

  $$\%N = (Ni / Nt) *100$$

  Where Ni is the total number of prey type "i", and Nt is the total number of prey items per predator.

- Percentage by reconstructed weight, %W:

  $$\%W = (Wi / Wt) *100$$

  Where Wi is the total biomass of prey type "i", and Wt is the total biomass of all prey items within the marine mammal species.

- The Index of Relative importance (IRI) was used to measure the importance of each prey species following Hyslop (1980). The use of the frequencies explained above might give a partial idea of the diet of the predators; a predator could prey on a large number of small fish, and percentage of biomass could be smaller than another predator that preys on small numbers of larger prey. The combination of this index gives a better idea of the importance of the different prey items in their diet:

  $$IRI = (\%N+ \%W) * \%F$$

  Where %F is the percentage frequency of occurrence of each prey, %N is the percentage of importance by number of each prey, and %W is the percentage of importance by weight.

To estimate the annual food consumption of the population is recommended to follow Pierce et al. (2011).

3.4.5 Plastic analysis

3.4.5.1 Digestion of the organic material

Reactives to be used to extract microplastics and digest organic material should be prepared in advance. The first extraction step can be carried out with two different reagents. Here we present the preparation of both:

- Potassium hydroxide (KOH): We should do 10% KOH solution adding 100g to 1,000ml of water. It is recommended to prepare the solution in a beaker or conical flask with a lid. Gently mix the pellets until dissolved. Leave until the solution turns cool. The final solution should be filtered using a Buchner filter or similar to ensure that there are no residual microplastics in the solution, either from the KOH pellets, water or from procedural contamination. This reactive is regularly used in areas of the Atlantic waters. The solution should be filtered to remove any plastic contamination.

- Hydrogen peroxide (H2O2): We should use 30% or 35% of H2O2. This reagent is regularly used in the Mediterranean Sea. The solution should be filtered to remove any plastic contamination.

The reagent should be poured in the glass jar with the sample and shake the jar gently to allow the sample to dissolve with the reagent. Note that it was mentioned before that the sample should have as litter water as possible to obtain the correct concentration of the solution for the sample. Different procedures will be carried out depending on the reagent to be used:

- KOH: the sample should be in the glass jar for three weeks to allow the digestion of organic material. To reduce the time, samples can be incubated at 60ºC for 24h with a continuous agitation (125rpm).
- H2O2: the sample will be incubated at 50-55ºC for 24h. If the organic material is not digested the sample should stay for longer periods.

After the period mentioned above, it should be noted that some of the sample may remain at the bottom of the jar and not dissolve. Feces contain a high amount of inorganic material (the remains in the jar), and density separation should be performed. Before beginning the density separation, the supernatant in the jar should be filtered for microplastic analysis.

The first density separation will be carried out using NaCl (1.2 g/cm³). NaCl should be added to a flask with water until saturation and filtered before use. The solution should be poured into the jar with the sample, at least 3-4 cm above the sample. The jar should be shaken gently to allow the sample to dissolve in the solution for several hours. Once the solution above the sample is clear, the supernatant should be filtered for microplastic analysis. It is advisable to repeat this procedure two or three times to recover all low-density microplastics from the sample.

The second density separation will be conducted using NaI (1.8 g/cm³). The same procedure used for NaCl will be followed. It is advisable to reuse the filtered NaI solution for other samples within the same set due to the higher cost of the reagent.

There is a third density separation, which is only recommended if the laboratory has the necessary facilities, resources, and personnel skilled in using the reagent. The reagent is SPT (2.8 g/cm³), and the procedure follows the same guidelines as the previous ones.

These guidelines can be found in Lusher & Hernandez-Milian (2018).

3.4.5.2 Filtration

The solution should be filtered using equipment such as a glass Buchner filter with a microfiber filter connected to a vacuum. If the laboratory does not have these facilities, filtration can be carried out using funnels with microfiber filters (e.g., coffee filters) as an alternative. The filtration should be performed in an area where airborne contamination is minimized or eliminated. Additionally, blanks should be placed around the working area. Note that if you are using a vacuum, some air currents will be produced nearby; therefore, avoid working close to the vacuum. Consider setting up a temporary divider or screen between the vacuum and the filtration setup, or conduct the filtration within a small, closed chamber.

Filters should be left to dry and stored in Petri dishes covered with aluminum foil to avoid the electrostatic energy that can cause the microplastics to move off the filter.

3.4.5.3 Visual characterization

The first step in identifying microplastics is to detect them visually. Filters will be inspected using a stereomicroscope with an attached camera to identify their color and size. It is recommended not to remove the filter from the Petri dish. This work should be conducted in a lab with the same restrictions as in previous steps. Following Lusher & Hernández-Milián (2018) and Lusher et al. (2014), visual characterization consists of the following steps:

- Measure the longest dimension (length and width)
- Record the shape categories: fiber, fragment, films, foams and spherical (beads). The division depends on the research.
- Record the colour. Keep in mind that colour is a subjective factor but it will aid in categorization.
- Check if particles are plastic following the next characteristics are homogeneous in color, no natural structures, they bend in an unnatural form, and in the case of the fibers, they should have a consistent

thickness throughout length and no fraying at ends of the fibers. Additionally, a hot needle can be used and in case of plastic will react to the heat through bending or melting.

3.4.5.4 Chemical characterization

Chemical characterization should be performed on a representative subsample. The Marine Strategy Framework Directive (MSFD) recommends analyzing 10% of the sample; however, as many particles as possible should be chemically analyzed to minimize identification errors.

Different specific instruments can be used for the chemical identification of polymers. The most commonly used instrument is Fourier-transform infrared spectroscopy (FT-IR), which performs a spectral analysis of the plastic and identifies the polymer based on a machine library. Microplastics should be dried prior to this analysis to avoid misidentification.

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5. Forms and Scales

Sea scale

Douglas scale	Definition	Height
0	Mirror calm	0 m
1	Slight ripples; no foam crests	0-0,1 m
2	Small wavelets; glassy crests, but not whitecaps	0,1-0,5 m
3	Large wavelets; crests begin to break; few whitecaps	0,5-1,25 m
4	Longer waves; many whitecaps	1,25-2,5 m
5	Moderate waves of longer form; some spray	2,5-4 m
6	Large waves; whitecaps everywhere; frequent spray	4-6 m
7	Sea heads up; white foam blows in streaks	6-9 m
8	Long, high waves; edges breaking; foam blows in streaks	9-14 m
9	High waves; sea begins to roll; dense foam streaks	>14m

Visibility scale

Visibility scale	Definition
0	Very low visibility
1	<1mile
2	1-3 miles
3	3-5 miles
4	5-10 miles
5	>10 miles

Wind scale

Beaufort scale	Definition	Wind speed	Sea conditions
0	Calm	<0.5 m/s [<1 mph]	Sea like a mirror
1	Light air	0.5-1 m/s [1-3 mph]	Ripples with appearance of scales are formed, without foam crests
2	Light breeze	1.6-3.3 m/s [4-7 mph]	Small wavelets still short but more pronounced; crests have a glassy appearance but do not break
3	Gentle breeze	3.4-5.5 m/s [8-12 mph]	Large wavelets; crests begin to break; foam of glassy appearance; perhaps faecestered white horses
4	Moderate breeze	5.5-7.9 m/s [13-18 mph]	Small waves becoming longer; fairly frequent white horses
5	Fresh breeze	8-10.7 m/s [19-24 mph]	Moderate waves taking a more pronounced long form; many white horses are formed; chance of some spray
6	Strong breeze	10.8-13.8 m/s [25-31 mph]	Large waves begin to form; the white foam crests are more extensive everywhere; probably some spray
7	High wind, moderate gale, near gale	13.9-17.1 m/s [32-38 mph]	Sea heaps up and white foam from breaking waves begins to be blown in streaks along the direction of the wind; spindrift begins to be seen
8	Gale, fresh gale	17.2-20.7 m/s [39-46 mph]	Moderately high waves of greater length; edges of crests break into spindrift; foam is blown in well-marked streaks along the direction of the wind
9	Strong/severe gale	20.8-24.4 m/s [47-54 mph]	High waves; dense streaks of foam along the direction of the wind; sea begins to roll; spray affects visibility
10	Storm, whole gale	24.5-28.4 m/s [55-63 mph]	Very high waves with long overhanging crests; resulting foam in great patches is blown in dense white streaks along the direction of the wind; on the whole the surface of the sea takes on a white appearance; rolling of the sea becomes heavy; visibility affected
11	Violent storm	28.5-32.6 m/s [64-72 mph]	Exceptionally high waves; small- and medium-sized ships might be for a long time lost to view behind the waves; sea is covered with long white patches of foam; everywhere the edges of the wave crests are blown into foam; visibility affected
12	Hurricane force	≥32.7 m/s [≥73 mph]	The air is filled with foam and spray; sea is completely white with driving spray; visibility seriously affected

Recording form for the collection of faecess (MS-MPs project)

(Please fill in as much as possible)

Name of recorders:

Date of sampling (dd/mm/yy): Location (Lat/long):

Cave number: Wind (Beaufort scale): Wind direction: Cloud (%):

Sea state (Douglas scale): Water depth (m): Visibility: Temperature (ºC)

Water: Air:

Human activities in the area:

Fisheries: Tourist:

Number of boats: Tourist boats: Yes ☐ No ☐ Num:

Small (<12m): Medium (12-24m): Large (>24m): Tourist close to caves: Yes ☐ No ☐

Type of fisheries: Divers: Yes ☐ No ☐

Presence of seals:
(Indicate if young, juvenile or adult)

Observations related to seals

Evidence of debris in the area:

Plastic: Yes ☐ No ☐ | No plastic: Yes ☐ No ☐
Num: | Num:

Evidence of debris in the cave:

Plastic: Yes ☐ No ☐ | No plastic: Yes ☐ No ☐
Num: | Num:

Number of faeces collected

Storage
Plastic: Foil:

Observations/Notes:

ANNEX II

Archipelagos
περιβάλλον και ανάπτυξη
environment and development

MEDITERRANEAN MONK SEAL

The No. 1 endangered marine mammal of Europe

By sharing your experiences you actively contribute to the conservation of the monk seal!
This information is extremely valuable. Personal data will be treated confidentially.
Thank you very much for your cooperation!

MONK SEAL OBSERVATION DATA

Observer's name:……..……………………………………….…....... Address:…………………………………………….…................

Occupation: fisherman / resident / researcher / tourist / sailor / other ……………………….………………

Observer's position: land / vessel / aquaculture / other…………………………………………………………………

Date of sighting: **20…./……./……..** Time**……..** Duration**……………..** Number of animals: ……………………………

Region's name: ………………………………………………………..………………………………… (land, island, off shore, etc.)

Precise location or position: ………………………………………… (cape, bay, beach) Closest town/village:…………………

ANIMAL No. 1 Photos/videos available? ………………………………………………………(*please send us a copy*)

At sea: approximate distance from observer: …………….. On land: inside cave / beach / rocky coast / other………

State and condition of the animal: normal / injured / ill / dead (corpse fresh / decaying) / unknown

Size class of the animal: up to 1,0 m 1,5 m 2,0 m 2,5 m 3,0 m unknown……………………………….

Colour: black / brown / dark grey / light grey / beige / whitish / unknown / other…………………………………….

Marks and description: spots / scars / patches / other ……………………………………………………………….……

Behaviour: swimming / diving / foraging / feeding / resting / sleeping / other………………….……………………

ANIMAL No. 2 Photos/videos available? ………………………………………………………(*please send us a copy*)

At sea: approximate distance from observer: …………….. On land: inside cave / beach / rocky coast / other………

State and condition of the animal: normal / injured / ill / dead (corpse fresh / decaying) / unknown

Size class of the animal: up to 1,0 m 1,5 m 2,0 m 2,5 m 3,0 m unknown……………………………….

Colour: black / brown / dark grey / light grey / beige / whitish / unknown / other…………………………………….

Marks and description: spots / scars / patches / other ……………………………………………………………….……

Behaviour: swimming / diving / foraging / feeding / resting / sleeping / other………………….……………………

In case you observed more than two animals together, for more details, for seals-fisheries interaction and for any other comments please use the space on the second page.

A r c h i p e l a g o s – environment and development * GR-28100 Lourdata * Kefalonia
Athens: Strofiliou 26 * GR-14561 Kifissia * e-mail: archipelagosgr@yahoo.gr
A r c h i p e l a g o s Italia, ambiente e sviluppo
Venezia, Calle Asiago 4 30132 Venezia e-mail: luigibundone@tiscali.it

Data on seals - fisheries interaction:

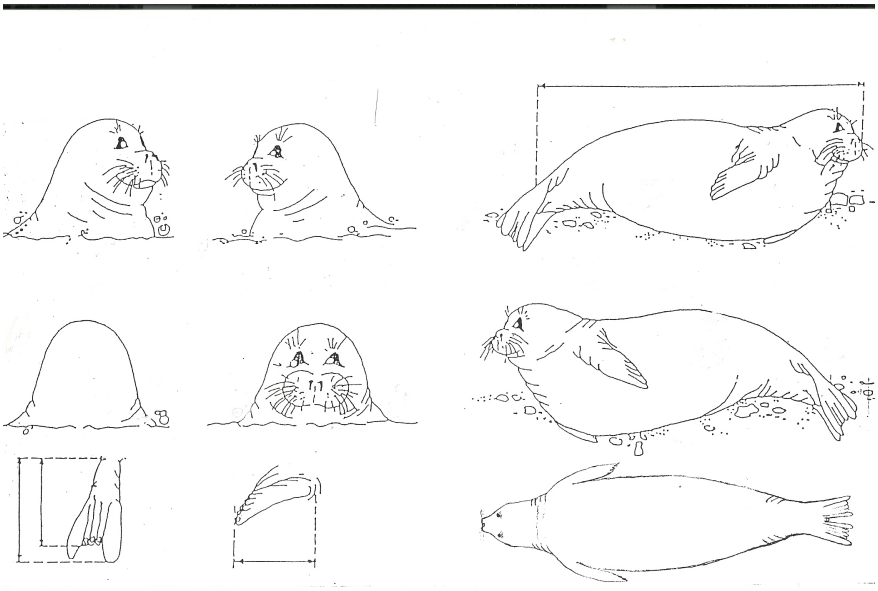
Type of gear: trammel nets / gill nets / bottom long lines / trawler / purse seines / other.....

Damage: YES / NO / unknown Seal(s) at the gear:.....

Fish eaten (species): .....

Gear damaged: .....

Please indicate detailed characteristics in the sketches by UNEP (size/colour/marks)

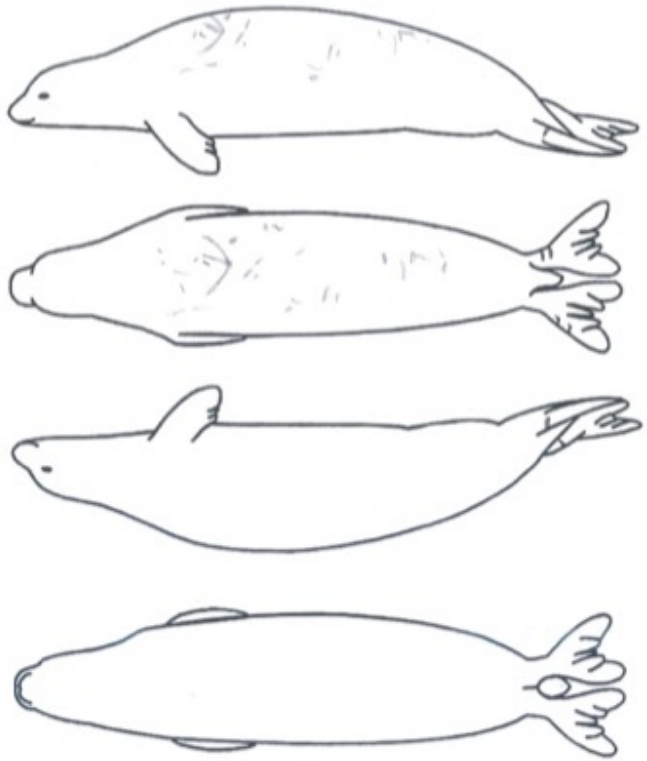


If possible, please draw a map of the sighting's location	Space for data about more animals sighted together

A r c h i p e l a g o s – environment and development * GR-28100 Lourdata * Kefalonia
Athens: Strofiliou 26 * GR-14561 Kifissia * e-mail: archipelagosgr@yahoo.gr
A r c h i p e l a g o s Italia, ambiente e sviluppo
Venezia, Calle Asiago 4 30132 Venezia e-mail: luigibundone@tiscali.it

ANNEX III

ODTÜ
METU



SEAL ID CARD

Name	: KITE
Region sighted	: Antalya
Sex	: male
Stage at first sight	: sub adult male
Cave used	: uca
ID features	: kite shape on dorsal
Reference photo	: uca_r_01060007