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Agenda item: Guidance on monitoring concerning the biodiversity and non-indigenous species common indicators

Monitoring protocols of the Ecosystem Approach Common Indicators 3, 4, 5 and 6

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Note by the Secretariat

The 19th Meeting of the Contracting Parties to the Barcelona Convention (COP 19) agreed on the Integrated Monitoring and Assessment Programme (IMAP) of the Mediterranean Sea and Coast and Related Assessment Criteria which set, in its Decision IG.22/7, a specific list of 27 common indicators (CIs) and Good Environmental Status (GES) targets and principles of an integrated Mediterranean Monitoring and Assessment Programme.

The list of agreed common indicators related to biodiversity and non-indigenous species cluster includes in particular:

1. common indicator 1: Habitat distributional range (EO1) to also consider habitat extent as a relevant attribute;
2. common indicator 2: Condition of the habitat's typical species and communities (EO1);
3. common indicator 3: Species distributional range (EO1 related to marine mammals, seabirds, marine reptiles);
4. common indicator 4: Population abundance of selected species (EO1, related to marine mammals, seabirds, marine reptiles);
5. common indicator 5: Population demographic characteristics (EO1, e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates related to marine mammals, seabirds, marine reptiles);
6. common indicator 6: Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species, particularly invasive, non-indigenous species, notably in risk areas (EO2, in relation to the main vectors and pathways of spreading of such species);

During the initial phase of the IMAP implementation (2016-2019), the Contracting parties to the Barcelona Convention updated the existing national monitoring and assessment programmes following the Decision requirements in order to provide all the data needed to assess whether ''Good Environmental Status'' defined through the Ecosystem Approach process has been achieved or maintained.

Decision IG.23/6 on the 2017 MED QSR (COP 20, Tirana, Albania, 17-20 December 2017) agreed, as general directions towards a successful 2023 Mediterranean Quality Status Report (2023 MED QSR), the following main recommendations:

(i) harmonization and standardization of monitoring and assessment methods;
(ii) improvement of availability and ensuring of long time series of quality assured data to monitor the trends in the status of the marine environment;
(iii) improvement of availability of the synchronized datasets for marine environment state assessment, including use of data stored in other databases where some of the Mediterranean countries regularly contribute; and
(iv) improvement of data accessibility with the view to improving knowledge on the Mediterranean marine environment and ensuring that Info-MAP System is operational and continuously upgraded, to accommodate data submissions for all the IMAP Common Indicators.

With the view to implement this recommendation, the present document provides information on the monitoring protocols of the agreed Ecosystem Approach common indicators 3, 4, 5 and 6 assess progress towards Good Environmental Status (GES).

The document is organized along 5 main monitoring guidelines:

(i) monitoring guidelines of cetaceans
(ii) monitoring guidelines of monk seals
(iii) monitoring guidelines of sea birds
(iv) monitoring guidelines of marine turtles
(v) monitoring guidelines of non-indigenous species

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A. Guidelines for monitoring Cetaceans in Mediterranean

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1. Introduction

1.1. Background

The Contracting Parties to the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention) have adopted the Ecosystem Approach (EcAp) in January 2008. This strategy allows all aspects of marine ecosystem to be taken into account. It includes management of coast, sea and living resources that promotes conservation and sustainable use in an equitable way, in order to respect interactions in the ecosystems. Indeed, it recognizes ecological systems as a rich mix of elements that interact with each other continuously. This process aims to achieve the good environmental status (GES) through informed management decisions, based on integrated quantitative assessment and monitoring of the marine and coastal environment of the Mediterranean. EcAp is also a way of making decisions in order to manage human activities sustainably. It recognizes that human's activities both affect the ecosystem and depend on it.

In February 2016, the Contracting Parties to the Barcelona Convention have also adopted an Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP). This text describes the strategy, themes and products to deliver by Contracting Parties over the second period of the implementation of the EcAp (2016-2021). The main goal of IMAP is to build and implement a regional monitoring system gathering reliable and up-to-date data and information on the marine and coastal Mediterranean environment. Mediterranean countries committed to monitor and report on 23 common indicators, articulated on 11 ecological objectives and covering topics related to pollution, marine litter, biodiversity, non-indigenous species, coast and hydrography.

One of eleven ecological objectives is “Biodiversity is maintained or enhanced” (EO1). Three determining factors are used to quantify the conservation:

1. no further loss of the diversity within species, between species and of habitats/communities and ecosystems at ecologically relevant scales;
2. any deteriorated attributes of biological diversity are restored to and maintained at or above target levels, where intrinsic conditions allow;
3. where the use of the marine environment is sustainable.

1.2. Aim of this doc

These guidelines aim at helping managers and decision makers to understand and implement a strategy of long-term monitoring for cetaceans, in deciding what kind of method to choose at regional and national level to answer the indicators 3, 4 and 5. This document aims at presenting a global overview of methods, with the main advantages and disadvantages, the human resources and material requested in order to better estimate the investment needed and other practical points. For more details on one specific method, please follow the bibliographic references.

A lot of scientific papers, or guidelines exist on the subject and on all those methods that are recognised as standard. Some explain in detail the steps of implementation, the scientific background, highlight also pro and cons, advantages and disadvantages. A list of some of these documents are listed at the end and should be considered for further details.

This document focuses more on the techniques at sea than on the consequent and associated analyses. It has to bear in mind that analyses need expert’s time and skills and has a certain cost related in order to be properly done. A lot of models and types of analyses exist and are well described in many scientific papers. What should be stressed is that powerful analyses can be led only with reliable data that have been collected in a standardised and recognised manner. So, to be sure data will be useful, comparable and used, the decision and implementation of rigorous methods should be the first step, following standard monitoring methods here highlighted.

1.3. Indicators 3, 4, 5

In the context of the Barcelona Convention, a common indicator is an indicator that summarizes data into a simple, standardized, and communicable figure. It is able to give an indication of the degree of threat or change in the marine ecosystem and can deliver valuable information to decision makers.

Among five common indicators related to biodiversity (EO1) fixed by IMAP, three are about marine mammals:

- Indicator 3 - Species distributional range

This indicator is aimed at providing information about the geographical area in which marine mammal species occur. It is intended to reflect the species distributional range of cetaceans that are present in Mediterranean waters, with a special focus on the species selected by the Parties. The main outputs of the monitoring under this indicator will be maps of species presence, distribution and occurrence. Resulting analysis can led also to identification of important habitat and core areas for the species. The aim is to detect any important changes in the distributional pattern of the cetaceans.

- Indicator 4 - Population abundance of selected species

As cetaceans are highly mobile and distributed mainly over vast areas, this indicator refers preferably also to an area-defined abundance of selected species (in a specified area in a given timeframe). Resulting analysis led to absolute abundance, density maps or indices of abundance. The aim is to detect any important changes in those numbers. Methods for estimating density and abundance are generally species-specific and ecological characteristics of a target species should be considered carefully when planning a research campaign. The main limitation of some implementation of monitoring method is relates to how representative the results are in terms of the relevant population. So, it needs first to define which population is targeted.

- Indicator 5 - Population demographic characteristics (e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates)

This indicator required to demographic parameters as the age structure, age at sexual maturity, sex ratio and rates of birth (fecundity) and of death (mortality). These data are particularly difficult to obtain for marine mammals and to monitor, but are important to understand and collect. Monitoring effort should be directed to collect long-term data series covering the various life stages of the selected species. This would involve the participation of several teams using standard methodologies and covering sites of particular importance for the key life stages of the target species. Results are in terms of numbers or rates. The aim is to detect any important changes in those numbers or ratio. One of the main limitations of some implementation of monitoring method is relates to how representative the results are in terms of the relevant population. So, it needs first to define which population is targeted.

2. Species concerned

IMPA fixes a reference list of species and habitats to be monitored. Among eleven species considered to regularly occur in the Mediterranean Sea, eight cetacean species are selected, divided into three different functional groups:

- Baleen whales: fin whale (*Balaenoptera physalus*)
- Deep-diving large dolphins: sperm whale (*Physeter macrocephalus*), Cuvier’s beaked whale (*Ziphius cavirostris*),
- Shallow diving small toothed dolphins: short-beaked common dolphin (*Delphinus delphis*), striped dolphin (*Stenella coeruleoalba*), common bottlenose dolphin (*Tursiops truncatus*), long-finned pilot whale (*Globicephala melas*), Risso’s dolphin (*Grampus griseus*),

The Contracting Parties, while updating their national monitoring programmes, shall make every effort to identify the list of species to be considered with at least two monitoring areas, one in a low-pressure area (e.g. marine protected area/ Specially Protected Area of Mediterranean Importance (SPAMI)) and one in a high pressure area from human activity. The choice will have to take into account on the specificity of their marine environment and biodiversity, and also on the number of animals occurring in the Contracting Parties' waters and how many there are in relation to total populations size to warrant investigating one or more of the indicators.

3. Monitoring methods

Before embarking upon a monitoring programme, the most important is to identify the objective, determine the appropriate indicator(s) in principle, then determine precisely what information can be gained and what are the limitations. Then a cost-benefit analysis of the various options available should be conducted. The type of platform, level of sophistication of survey, and detection method should be considered in each case, and the most appropriate ones identified, relying upon if the indicator can be monitored to be able to robustly detect changes should they occur given certain levels of effort (sample size).

Thus, when being in the process to decide which monitoring method to be implemented, it is important to consider several issues, that will be synthetized in different tables to get a global first overview. General consideration will give some advices considering on unifying data collection protocols and the statistical requirements on data and samples, and also the complementarity of methods at different spatial and temporal scales, as no single method will be enough to monitor all parameters and all species. The other chapters will present more in details the different methodologies.

Methods for estimating density and abundance are generally species-specific and ecological characteristics of a target species should be considered carefully when planning a research campaign.

3.1. Synthesis tables

Four tables synthetized the main information needed to take the decision on what method(s) to implement to elucidate indicator 3, 4 and 5 of the EO1 of the IMAP process:

- which method will give useful data to answer which indicator, depending on the target specie(s) and its characteristics. This is presented in a synthetic way in Table 1 for an overview;

- according to the method chosen, what is the time delay to get results, what is the cost associated, what is the difficulty in implementing the method, the constraints and limits associated and finally the compatibility with other method(s) (in order to optimize time and resources, as several methods can be used in parallel on the same platform during the same campaigns). These indications are gathered in Table 2.

- according to the method chosen, what will be the investment needed, in terms of material and human resources (Table 3)

- according to the level at which there are designed for, population or individuals, and at which spatial scale they correspond the best (small or large area). In Table 4 each method has been designed to collect data to answer question at one of the levels and spatial scales, whereas some adaptation can be made to other level and spatial scale. Additionally, some methods are designed for large areas and the platform will have to move within the large areas. Whereas some methods, especially the one based on individuals, will be implemented in small areas and can give information on large areas in two ways: if the implementation is done in several places and built in a frame of a network (e.g., strandings, photo-ID), or by the nature of the parameter studied which can be extrapolate in a wider area if enough samples are available (reproductive status, genetic, telemetry).

Finally, as working at sea can be expensive and as marine environment and IMAP process deal also with other marine species, Tableau 5 presents the monitoring methods for cetaceans and their compatibility with other marine species monitoring.

Table 1 - Synthesis listing different cetacean's monitoring methods recommended answering to indicators of IMAP process by cetacean species (legend: bold type = best suitable method; in bracket (less suitable method but can give interesting information) and in bracket and italic (*indication of limits*)). For the definition of the methods, see other chapters of the document.

	Baleen whales	Deep-diving toothed whales		Other toothed whales				
	fin whale (*Balaenoptera physalus*)	**sperm whale (*Physeter macrocephalus*)**	**Cuvier's beaked whale (*Ziphius cavirostris*)**	**long-finned pilot whale (*Globicephala melas*)**	**Risso's dolphin (*Grampus griseus*)**	**common bottlenose dolphin (*Tursiops truncatus*)**	**striped dolphin (*Stenella coeruleoalba*)**	**short-beaked common dolphin (*Delphinus delphis*)**
INDICATOR 3, species distributional range	**Visual Line transect boat or aerial** Telemetry Acoustic line transect (or fixed point) (*presence/absence*) Land based method (*locally*)	**Visual Line transect boat coupled to acoustic line transect** Photo-Identification Telemetry (Visual Line transect aerial)	**Visual Line transect boat coupled to acoustic line transect** Telemetry and acoustic fixed point Photo-Identification (Visual Line transect aerial)	**Visual Line transect boat or aerial** Acoustic line transect (or fixed point) (*presence/absence*)	**Visual Line transect boat or aerial** Photo-ID Acoustic line transect (or fixed point) (*presence/absence*)	**Visual Line transect boat or aerial** Photo-Identification Acoustic line transect (or fixed point) (*presence/absence*) Land based method (*locally*) (By-catch)	**Visual Line transect boat or aerial** Acoustic line transect (or fixed point) (*presence/absence*) (By-catch)	**Visual Line transect boat or aerial** Acoustic line transect (or fixed point) (*presence/absence*) (By-catch)
INDICATOR 4, species population abundance	**Visual Line transect boat or aerial** Acoustic line transect (*indices of relative abundance*) Photo-identification	**Visual Line transect boat coupled to acoustic line transect** **Photo-Identification**	**Photo-Identification** **Visual Line transect boat coupled to acoustic line transect**	**Visual Line transect boat or aerial** Acoustic line transect (*indices of relative abundance*)	**Photo-Identification** **Visual Line transect boat or aerial** Acoustic line transect (*indices of relative abundance*)	**Visual Line transect boat or aerial** **Photo-Identification** Acoustic line transect (*indices of relative abundance*)	**Visual Line transect boat or aerial** Acoustic line transect (*indices of relative abundance*)	**Visual Line transect boat or aerial** Acoustic line transect (*indices of relative abundance*)
INDICATOR 5, Population	**Photo-identification** Biopsy	**Photo-identification**	**Photo-identification**	**Photo-identification**	**Photo-identification**	**Photo-identification**	Biopsy Stranding	Biopsy Stranding

demographic characteristics	Stranding By-catch	Biopsy Stranding By-catch	Biopsy Stranding By-catch	Biopsy Stranding By-catch	Biopsy Stranding By-catch	Biopsy Stranding By-catch	By-catch	By-catch

Table 2- Synthesis for the different cetacean's monitoring methods concerning which indicators of the IMAP process they may help with, the time delay to obtain results, their cost, the level of constraints associated, their limits or bias and an indication concerning the compatibility among methods. + = low, +++ = high.

METHOD	INDICATOR	RAPIDITY OF RESULTS	COMPATIBILITY WITH OTHER METHODS	COSTS	CONSTRAINTS	LIMITS
Visual Line transect boat	3- distributional range 4- abundance	Short-term	**acoustic line transect** (sometimes photo-Identification if approaching mode)	+++	+++	Bias due to responsive movements of animals; detectability to be assessed,
Visual Line transect aerial	3- distributional range 4- abundance	Short-term		++++	++++	For deep diving species the number of sightings will be too low to give reliable results.
Photo-identification	3- distributional range 4- abundance 5- demographic characteristics	Can be medium-term but is far more reliable on long-term	**biopsy and telemetry** (sometimes line transect boat, depending if approaching mode)	++	++	Only applicable for species with long-lasting individual identifiable natural marks.
Land based method	3- distributional range 4- abundance	Short-term and long-term	acoustic fixed point, (photo-Identification depending on conditions)	+	+	Limited to small detection area and suitable coastal landscape.

METHOD	INDICATOR	RAPIDITY OF RESULTS	COMPATIBILITY WITH OTHER METHODS	COSTS	CONSTRAINTS	LIMITS
Acoustic line transect	3- distributional range 4- abundance	Short-term	**visual line transect**	+++	+++	Relies upon animals being vocal.
Acoustic fixed point	3- distributional range 4- abundance	Short-term	land based method (if near coast)	++	+	Relies upon animals being vocal. Low spatial resolution or need a network of several hydrophone, and logistical problems with deployment.
Telemetry	3- distributional range	Short term Long-term	**biopsy and photo-Identification**	+++	++++	Only allows small samples resulting in much inter-individual variation. Invasive.
Biopsy	5- demographic characteristics	Long-term	**photo-Identification, telemetry**	++	+++	Invasive method. Requires large sample size.
Stranding	3- distributional range (4- abundance) 5- demographic characteristics	Short- and long-term		+	+	Efficient if networking is implemented.
By-catch	3- distributional range 5- demographic characteristics	Short- and long-term		+	+	Efficient if special observers are involved, or a reporting well established program is implemented by Fisheries Agency
Unmaned Autonomous vehicle (drone and submarine AUV)	3- distributional range 4- abundance	Short- and long-term		++++	+++	Method in development.
Pictures and video	3- distributional range 4- abundance	Long-term	line transect aerial	++	+++	Method and technic in test, not standardised yet.

Table 3- Synthesis for the different cetacean's monitoring methods about the material and human resources involved and the level of skills needed

METHOD	MATERIAL NEEDED Color legend : in black "investment" ; in orange "operational "	PLATFORM	MINIMUM N. OF PERSONS NEEDED	SKILLS
Visual Line transect boat	- binoculars - GPS, watch - instruments to estimate or measure the distance of the animals from the boat (reticulate binoculars, measuring stick) - observation forms or computer or mobile phone - corner quadrants or angle board	Vessel dedicated (like motor or sailing boat) or not dedicated ("fix line" like ferries or oceanographic vessels)	4	++
Visual Line transect aerial	- observation forms or computer with a person to enter data in real time, or dictaphone - clinometer - GPS, watch	Airplane small, high-wing, that can fly slowly while remaining within the limits of safety, equipped with bubble windows (to allow the observer to look "outside" of the airplane to look under it) and can carry at least three people (two observers and a data recorder).	3 + pilot	+++
Photo-identification	- observation forms or computer or mobile phone - GPS, watch - camera with lens	Vessel small or relatively small boat (outboard or an average zodiac boat) with a sufficiently low bridge over the water to take pictures at the correct angle.	1 (3)	+
Land based method	- binoculars or telescopes - observation forms or dictaphone or computer - watch - theodolite or clinometer camera for photogrammetry - Compass or quadrant angles or angle boards	Land	1 (2)	++
Acoustic line transect	- binoculars - GPS, watch - observation forms - hydrophone coupled to stereo amplifier - sound-recording instrument and power source	Vessel Irrespective of the type, which is able to hold a constant speed and a course for use in transect. Preferably silent.	1 (2)	+++

METHOD	MATERIAL NEEDED Color legend : in black "investment" ; in orange "operational "	PLATFORM	MINIMUM N. OF PERSONS NEEDED	SKILLS
Acoustic fixed point	- binoculars - GPS, watch - observation forms - hydrophone coupled to stereo amplifier - sound-recording instrument and power source	Beacon, buoy Or vessel	(1)	+
Telemetry	- beacon - crossbow or long pole	Vessel	1 (2)	++
Biopsy	- crossbow or gun and bolts - storage and cleaning material - freezer/frozen storage	Vessel small or relatively small boat (outboard or an average zodiac boat) with a sufficiently low bridge over the water to shoot at the correct angle.	1 (2)	++ Need specific skills
Stranding	- stranding forms - camera - tape measure - sampling kit (knife, shears, packaging materials) - dedicated dress, safety gloves, safety glasses - freezers - fixing solution such as formalin, ethanol, DMSO	Land	1	++ Need to make sure this is handled by a trained and authorized scientist or veterinary
By-catch	- GPS, watch - observation forms - camera - tape measure - sampling kit (knife, shears, packaging materials)	Vessel	1	+
Unmaned Autonomous vehicle (drone and submarine AUV)	- drone or submarine AUV	Vessel	1 (2)	+++ Need specific skills
Pictures and video	- high resolution camera	Airplane	(1) + pilot	++

Table 4 – Characteristics of cetacean's monitoring methods in regard to indicator 3, 4 and 5 of the IMAP process : at which level they are implemented (population or individuals) and at which spatial scale they correspond the best (small or large area). The darker the color, the best suited characteristics and the lighter the color, the more adaptation you have to implement this method for that area or level. Method implemented on individuals can be designed (network, large samples size) in order to give results at the population level (for indicator 5). In cells is given an indication of the time frame and frequency of the campaigns implementing the described methods at the corresponding spatial scale.

Cetacean monitoring method	Population level	Individual level	Large area	Small area
Visual Line transect dedicated boat			1 or 2 / 10 years	Yearly or seasonal
Visual Line transect dedicated aerial			1 or 2 / 10 years	
Visual Fix line transect by ferry or oceanographic vessel			Yearly, seasonal or monthly	
Acoustic line transect			1 or 2 / 10 years	Yearly or seasonal
Dedicated observers on opportunistic platform			Yearly or seasonal	Yearly or seasonal
Photo-identification	X		(network) Yearly or several years	Yearly or seasonal
Telemetry				
Biopsy	X			
Land based method				Yearly or seasonal
Acoustic fixed point	X		(network)	Yearly or seasonal
Stranding	X		(network)	Seasonal, monthly
By-catch	X		(network)	Seasonal, monthly

Tableau 5 - Compatibility with other species monitoring for the indicator 3, 4 and 5

Cetacean monitoring method	Seabirds at sea	Turtles at sea	Sharks	Other big fish (tuna, sunfish, swordfish, ray)	Floating Marine Litter
Line transect dedicated boat	X	X	X	X	X
Line transect dedicated aerial	X	X	X	X	X
Fix line transect by ferry or oceanographic vessel	X	X	X	X	X
Dedicated observers on opportunistic platform	X	X	X	X	X
Photo-identification surveys	X	X	X	X	X
Land based method	X	0	0	0	0
Acoustic line transect	0	0	0	0	0
Acoustic fixed point	0	0	0	0	0
Telemetry	X	X	X	X	0
Biopsy	X	X	X	X	0
Stranding	0	0	X	X	0
By-catch	X	X	X	X	X

3.2. General considerations

3.2.1. Scientific consideration on sampling and analysis

To ensure that the chosen method and the study design will be able to provide data to answer to the question posed with a useful level of precision, a power analysis should be run. It is useful to use existing data if any during this step. And the power analysis helps in indicating the ability of the statistical procedure and the available or planned data to reveal a certain level of change i.e. the ability to detect a trend of a given magnitude. Concretely the power analysis will help to plan studies to calculate the necessary sample size (e.g. the length of time series of abundance estimates), or the coefficient of variation (CV) of those estimates.
The use of existing software programs, as "TRENDS" (freely available at (https://swfsc.noaa.gov/textblock.aspx?Division=PRD&ParentMenuId=228&id=4740) helps greatly in the process.

But as cetacean's species are highly mobile, spread over vast areas which led to difficulties to cover the whole population or their whole range, another method to increase power to detect trends is to design a trend-site survey design. This site is sought to maximize precision by focusing on a smaller area to survey and increased the effort in the chosen area. The smaller area could correspond to a representative part of the range of the stock or to a stock identified at a smaller spatial scale as demographically independent populations. Finally, one of the most common methods to increase our ability to detect precipitous declines are to increase survey frequency (annual for example). Other useful methods are tested, more during the analysis, as to change the statistical decision criterion.

Many of the methods here described work under certain assumptions (equal coverage, homogeneity of capture, detectability, etc) and a great care should be taken in dealing with these assumptions since the beginning of the implementation. Associated data should be collected in order to calculate the correction factors if needed.

3.2.2. Complementarity of monitoring methods

There is an interest in **implementing several methods, as they can be complementary in spatial or temporal scales and for the different species**. This should be defined case by case, according to the objectives, the species, the area and the means (human resources, platform and funds). As the objective of monitoring population of cetaceans is to **detect trends** over time, it has then to be considered to choose one or several methods and to **plan to implement campaigns on a regular basis** in order to get several results over time. Often, large-scale dedicated campaigns are more expensive than non-dedicated campaigns or small-scale campaigns. For example:

- **a large-scale** (the whole waters under national jurisdiction of a country at least, entire basin, entire seas) **visual line transect dedicated survey** made with a vessel or an airplane will give you insights of abundance and distribution of several visible and numerous species (whales and delphinids). In the meantime, if the campaign is boat-based, you can add a hydrophone to the vessel to collect **passive acoustic** data on abundance, distribution and presence/absence of deep diving species (sperm whale, Ziphiidae). As those large-scale dedicated campaigns might be one of the most expensive ones, they are often implemented at least once or twice per decade.

- In parallel **non-dedicated vessel - or aerial- based line transect surveys** should be implemented to get data and results on a yearly basis (with one or two samples a year for oceanographic campaigns, even one sample per month for ferry). This will allow you to know inter-annual variability (year with typical, rich or poor abundance) and to correct the results of your dedicated large-scale survey the year it is implemented.

- When an important or representative **smaller area** is defined (MPA, Important Marine Mammal Are, etc), based on the results of this/these previous large surveys, you can implement **visual and acoustic line transect surveys in this small representative area**. Ideally, seasonal monitoring programmes should be conducted at this scale (at least during winter and summer periods).

- And finally, you can focus on some species and launch **individual-based tracking**, implementing photo-identification, biopsy and/or telemetry programmes. Those methods are highly complementary to the previous ones.

3.2.3. Trained and qualified personal

These methods are rigorous and high quality designed, implementing standard protocols and awaiting standard data. So, **people** implementing one of these methods at sea **should be trained to acquire the requested skills and knowledge** to do it in the correct way. If necessary, funds for training must be included in the program's budgets.

3.3. Standard Monitoring methods of living animals

3.3.1. Visual monitoring method

For visual surveys, it is important to consider observer skill and experience. Observers may vary in sighting efficiency; hence, training is important to obtain consistent results in species identification, counting of individuals and measuring information (distance, angle, time of diving…). An observer training must be scheduled upstream to visual monitoring campaigns.

3.3.1.1. Line transect method

In line transect sampling, a survey area is defined and surveyed along a sampling design of pre-determined transects ensuring equal coverage of the area. The perpendicular distance of each detected animal to the transect is measured and consequently used to obtain a detection function, from which an estimate of the effective width of the strip that has been searched can be calculated. Abundance is then calculated by extrapolating estimated density in the sampled strips to the entire survey area. The calculated number is therefore an estimate of abundance in a defined area at a particular time with its uncertainty. Assumptions relating to detectability and responsiveness need to be addressed and various methods (such as two-platform surveys) have been developed to accommodate these.

This method, either boat- or aerial-based, is mainly used to collect data in order to answer to abundance and distribution questions on cetaceans (indicator 3 and 4). When the platform is dedicated to the mission of collection of data on cetaceans, the whole process of implementation is better robust, namely quantity of effort, equal coverage with dedicated sampling design, bias on detectability, etc. When observers go aboard a non-dedicated platform, the data collection may be less designed to provide all necessary data to ensure a robust results and data to detect trends. Unless the routes and effort covers the whole area and the effort is frequent and regular (ex : ferry routes in the Pelagos Sanctuary, or fishery campaigns covering the whole Gulf of Lion each summer in 10km space transects). Finally, observers on opportunistic platform collect interesting and complementary data that can be less robust to answer to the indicators. But this has to be assessed in a case by case cost-benefice study, as in several occasions, something interesting can be launch with existing platforms.

3.3.1.1.1. Dedicated boat-based survey

Principle

Systematic surveys carried out from a boat constitute a powerful method primarily aimed at assessing the abundance and distribution of cetacean species over large areas. The boat follows a path corresponding to a predefined sampling plan, which covers the area of study as homogeneously as possible and records all cetacean sightings. The minimum amount of effort required to perform the analysis depends on the density of animals in the study area. The amount of effort must be calculated before designing the sampling plan. Often it is required that at least 40 sightings of one species is needed to get reliable results with lower uncertainties. To cope with assumptions (detectability and responsiveness), often a two-platform surveys is implemented, corresponding to two different teams of observers working independently of each other on the same platform if possible. Comparing their specific data helps in correcting the bias.

Human resources

The Line transect method required that 180° in front of the vessel is continuously observed during all daylight hours. This required that at least two trained observers are watching at all time, and to allow resting and meal time, it is required at least two teams rotating each two hours. So, for long lasting mission, 4 trained observer is a minimum, the best option is at least 3+3 allowing a better coverage and a person also dedicated to record the sightings and all associated information.

For double-platform then, a supplementary team of 3 observers is requested.

Material needed

- A boat with the required characteristics to carry out the mission for the planned duration, the survey area and the desired collection protocol.

- Binoculars (and for double-platform, a high-power ("big eyes") binoculars on a tripod or other support).

- Compass or angleboard.

- Instruments to estimate or measure the distance of the animals from the boat (reticulate binoculars or a video camera for photogrammetry, or measuring sticks or ruler, etc.).

- Observational forms and a computer.

- A watch.

- A GPS.

Implementation

The first phase is the preparation of the campaign, with training of people if needed, design the sampling scheme according to densities of cetaceans (if known) and habitats. Also, everything concerning authorization request and logistic should be considered largely before.

Effort should be precisely known, so start and end are recorded. During effort, observers scan the water for cetaceans while the vessel steams along predetermined transect lines at constant speed and heading. Often the speed is at 10 knots for large vessels, but it can be 8 or 6 knots for smaller vessels. The speed should be higher to cetacean's speed in order to avoid re-sighting of the same group. When cetaceans are seen, the observers record data such as the species, location (latitude and longitude) of the encounter, general behavior of the animals, and estimates of the number of cetaceans in the group. The sighting data are later analyzed using distance sampling statistical models and imported into a Geographical Information System (GIS) for further spatial analysis.

This method is reliable when wind, sea state and visibility are adequate to detect small dolphins, and the limit if often put to sea state and Beaufort wind less or equal to 3.

This type of monitoring may require some authorizations procedures, depending on study area (environmentally protected zones, cross border areas).

Advantages

- Allow representative coverage of areas.

- Different types of sample designs are available according to the characteristics of the study area and the census itself. The design of the sampling plan can be done using software DISTANCE (http://www.**distancesampling**.org).

- Protocols for data collection are standard and widely used; they are tested and improved continuously.

- Analytical methods are also standard, tested and constantly improved in order to minimize the influence of potential biases.

- Often, large vessels are required to cover large areas (vessels can remain at sea for many days, which can stay on course and maintain speed regardless of the sea state and can board sufficient personnel to allow rotation of the observer teams and secretaries). However, this method can also be applied to small areas with smaller boats (sailing vessels, motor boat).

Limitations

- This method is expensive, labor-intensive and give little spatial coverage.

- Applicable only in "good" weather conditions and by daylight.

- Responsive species movement prior to detection (i.e. attraction to, or avoidance of, the vessel) is difficult to predict but can generate substantial bias in estimates of abundance if it occurs.

- Theoretically, the line transect should not be interrupted: the boat must be "passage" mode, that is to say, it does not stop or turn away, which could lead to potential biases. Therefore, species identification and counting of individuals in groups can sometimes be difficult and it is incompatible with the collection of ancillary data, such as photographs for photo-identification, biopsies. It may be possible to make a part of the sampling plan in the "approach" mode where groups of easily identifiable and countable cetaceans are then approached before resuming the transect path. In this case, it is important to estimate the bias introduced in the protocol by this manoeuver and preserve it for conditions with real difficulties.

3.3.1.1.2. Dedicated aerial-based survey

Principle

Working by aerial means (airplane, helicopter) is a powerful method, primarily aimed at assessing the abundance and distribution of marine species over large areas or areas inaccessible by boat (far offshore area, harsh weather conditions, etc.). The platform used in most cases is a small airplane with one or more observers aboard. The airplane follows the path of a predetermined sampling plan to cover a large area as seamlessly as possible, noting all cetacean sightings. This technique can be aided by taking photographs or videos.

Human resources

At least 3 trained "aerial" observers should constitute the team in one airplane, 2 observers and 1 real time data recorder.

Material needed

- A small, high-wing airplane with two motors, that can fly at 90 knots while remaining within the limits of safety and for a duration of at least several hours. The airplane must be equipped with bubble windows (to allow the observer to look "outside" of the airplane and to look under it) and can carry at least three people (two observers and a data recorder) beside the pilot.

- Observation forms and ideally a computer with a person to enter the data reported by observers in real time, or a dictaphone.

- Two clinometers, one for each observer.

- printed angleboards

- A watch.

- A GPS

- A computer with dedicated maps and software.

Implementation

The first phase is the preparation of the campaign, with training of people if needed, design the sampling scheme according to densities of cetaceans (if known) and habitats. Also, everything concerning authorization request and logistic (localisation of airports, availability of fuel) should be launch largely before.

The pilot of the plane is in charge of following the flight plan defined and surveyed along pre-determined transects. Two observers sit at the bubble windows on the left and right side of the plane scan the water for cetaceans. And another scientist, the navigator, sit in the front at the co-pilot seat, is responsible for the flight plan too, entering effort data, environmental conditions and sightings data in real time into a laptop during the flight. When cetaceans are seen, the observers record data such as species, estimated group size and angle perpendicular to the trajectory of the airplane. The sighting data are later analyzed using distance sampling statistical models and imported into a Geographical Information System (GIS) for further spatial analysis.

This type of monitoring required a lot of authorization procedures specifics to aviation, in particular in cross border areas and also concerning airport use and fuel availability.

Advantages

- This technique is usually more profitable than large surveys over large areas, which would be conducted from the boat.

- Large areas can be covered in a short time and remote areas are reached quickly to study them (although the distance depends on the autonomy of the aircraft).

- Some sea conditions, such as waves, interfere much less when working from the airplane than from a boat.

- Provide opportunities to detect wildlife in real time and refine species identifications using a circle-back approach.

- The movement reaction issue (avoidance or attraction) is generally non-existent (if the aircraft is high enough and passes only once).

Limitations

- Visibility must be excellent (good sea conditions, clear sky, no glare, etc.) so flights are possible only on half (or less) of days available.

- There are difficulties in identifying species, and counting and detecting large groups of young cetaceans due to the altitude and / or speed of the aircraft, which allow only few seconds to the observers to collect all the data.

- A large component of availability bias exists due to the high speed of the aircraft.

- Sometimes the availability of appropriate aircraft characteristics (slow flight, high wings, sufficient autonomy, etc.) is rare.

- Data collection by air is expensive, particularly in remote regions away from airports.

- This technique is ineffective at capturing organisms that stay submerged for long periods like deep diver species.

- Aerial surveys are logistically difficult to implement, and incur high costs from aircraft hire and staffing and can be limited by flight regulations and safety considerations.

3.3.1.1.3. Not dedicated boat-based survey, or Fix line transect by ferry or regular oceanographic vessel's campaigns

Principle

Surveys are conducted along fixed transects using passenger ferry or oceanographic vessels as platform of observation. Teams of trained marine mammal observers (MMO) board either a passenger ferry which conducted almost identical transects from month to month or an oceanographic vessel conducting regularly the same design over the same area (for example yearly national small pelagic fish stock assessments campaigns). Data collection of occurrences of marine mammals are conducted on "passage" mode, that is to say, it does not stop or turn away. The method implemented is the line transect and the purpose of the method is to repeat the same transects in the long-term.

On those kind of vessel, reliable data on distribution and abundance can be collected, depending on the type of routes and regularity of crossing. For example, in the Pelagos Sanctuary, the ferries run almost all year round, on numerous routes crossing the whole area, ensuring a good spatial and temporal coverage. Also, oceanographic small fish stock campaigns often follow a tied coverage of their area of interest. Those data may be of great interest to answer to indicator 3 and 4 in those conditions.

Human resources

The Line transect method required that 180° in front of the vessel is continuously observed during all daylight hours. This required that at least two trained observers are watching at all time, and to allow resting and meal time, it is required at least two teams rotating each two hours. So, for long lasting mission, 4 trained observers is a minimum, the best option is at least 3+3 allowing a better coverage and a person also dedicated to record the sightings and all associated information.

Material needed

- Passenger ferry using fixed lines allowing repetitions or oceanographic vessel implementing on a regular basis the same (or equivalent) design in the same area

- Binoculars.

- Compass or angleboards

- Instruments to estimate or measure the distance of the animals from the boat (reticulate binoculars, measuring sticks and clinometer).

- Observational forms and a computer.

- A watch.

- A GPS.

Implementation

Observer's team conducted the survey from the deck of engine control room of the vessel or outside in a free of obstacle's observer point. They are divided on each side of the ferry/oceanographic vessel and collect data of cetacean's occurrence continuously on both sides. When "on effort", they scan carefully the area (with a focus on the 180° to the front of the boat) by eye and using binoculars, so as to detect visually cetaceans present on surface.

This type of monitoring required some agreements with ferry companies/oceanographic/fishery institutions.

Advantages

- This method, in a representative sector, gives relevant indicators of what occurs surroundings (in terms of distribution and indices of abundance).

- It is a cost-effective means of providing wide coverage over protracted periods. Furthermore, the use of these platforms allows to realize a monitoring all year round or yearly and at a lower cost.

- The regularity with which the crossings are made allows to repeat the operation as much as desired to refine a study.

- in some areas, ferry routes make a kind of sampling design relatively tied, allowing a good coverage of the area (ex.: Pelagos Sanctuary), and also oceanographic small fish stock campaigns often follow a tied coverage of their area of interest.

Limitations

- The major limitations are that there is rarely any control over the routes taken which are already designed, nor the speed of the vessel, and the vessel typically cannot divert from its track to confirm species identity or group size.

- Sometimes the required number of even only 2 observers cannot be allowed aboard, depending on the size of the vessel

- The application of this method is strictly speaking incompatible with the collection of ancillary data focusing on individual animals, such as photographs for photo-identification or biopsies.

3.3.1.1.4. Dedicated observers on opportunistic platform (military, custom, navy, whale-watching boats)

Principle

One or more observers board an opportunistic platform and benefit from the platform route to make observations without logistical implementations. Platforms can be boat-based or aerial-based.

Ideally, the effort should be significant to obtain a large number of observations and cover as homogeneously as possible the different values used in the environmental variables analysis. So, the platform should go at sea on a regular basis, and within the same area to be of some interest for monitoring objective of distribution and indices of abundance. So, military or custom's vessel, airplane or helicopter can be targeted, as well as whale-watching boats.

This method, not dedicated to cetaceans studies, are less robust to answer to the assumptions needed to get reliable and precise results in terms of indicator 3 and 4. Nevertheless, the fact that the same area is regularly sampled in the same way allows to gain knowledge on occurrence, presence and even indices of abundance and moreover, to compare these results between seasons and years.

Human resources

Depending on method implemented, size and authorization of the platform, at least 1 trained observer is required, and the higher the number of observers, the higher the quality of visual coverage and data recording.

Material needed

- Binoculars.

- Compass or angleboards

- Instruments to estimate or measure the distance of the animals from the boat (reticulate binoculars, measuring sticks, clinometer).

- Observational forms and a computer.

- A watch.

- A GPS.

Implementation

Observers team conducted the survey and scan carefully the area, with a focus on the 90° to the front of the boat, and with a focus below and perpendicular to trackline for aerial platform. Searching visually cetaceans present on surface has to be done by eyes and binoculars are used to precise parameters such as species, numbers, etc. During every observation period they record the begin and end of effort, the environmental condition and sightings data such as species, estimated group size, behavior, GPS location. Depending on the platform and its mission, ancillary data may be possible to collect.

This type of monitoring required some agreements with other structures.

Advantages

- Platforms of opportunity are often used to survey areas at low cost. In some cases, costs may be relatively small because boats and equipment can be minimized without compromising the reliability of the results of a simple, but adequate data collection protocol.

- Data collected from an opportunistic platform can still be used to assess habitat use and to estimate the abundance of animals through spatial modelling. In addition, the use of environmental characteristics to estimate abundance or relative abundance can potentially increase the accuracy of results. Finally, some platforms allow photo-identification or acoustic data to be taken.

Limitations

- The major limitations are that there is rarely any control over the routes taken, the speed of the vessel, the ability of vessel to divert from its track to confirm species identity or group size and even to take ancillary data (photo-identification). But this may vary greatly depending on the type of platform and mission.

- Monitoring implementation can be a low priority in initial objectives of the platform.

- The use of this kind of data should be done carefully, because there might exist a lack in the sampling design with uncovered area, heterogeneity in effort coverage across the range of values for the explanatory variables, …

- area covered might be small and unrepresentative for cetaceans

3.3.2. Passive acoustic monitoring

All cetaceans produce sounds like “clics” for echolocation or “whistles” (frequency modulated sounds) for intraspecific communication. Passive acoustic methods allow the near-continuous detection and monitoring of those sounds. The monitoring of these sounds allows for the collection of information on spatial and temporal habitat use, as well as estimation of relative density for some species and even abundance for sperm whale.

3.3.2.1. Passive Acoustic “line transect” (towed hydrophone)

Principle

One array with at least two hydrophones are towed by a moving boat. Listening and recording can be continuous or by samples. The array enables to determine angle at perpendicular distance, which is the base of the analysis of the “line transect” method. The trajectory of the boat should be constant in speed and heading, following a predefine design or random transects.

The area covered is bounded by the probability of detection by the hydrophone and the frequency and power of the sound made by the animals.

This is the most effective method to survey sperm whale, as they are long-deep diving species, and they use "clics" during the entire duration of their dives. Acoustic data from sperm whales can be used to assess both relative and absolute abundance and also distribution, provided that the appropriate equipment and survey design is followed. For other species, acoustic results might be complementary to visual for indicator 3, but not for indicator 4 as methods to relate sounds to abundance of animals are not efficient yet.

Human resources

At least one passive acoustic operator is needed, or more for a 24 hours work.

Material needed

- A boat, motor or sailing one, which is able to hold a constant speed and heading for a transect and be silent or can stop the engine often (for sampling).

- A whole acoustic acquisition chain:

    - hydrophone array composed of at least two hydrophones (even two arrays of hydrophone) coupled to stereo amplifiers and which is within a pipe that can be towed.

    - A DAQ system (convert the signal from analog to digital format and also convert in quantization)

    - A computer with a software analyzing sounds.

    - and a power source to power the system

- The relevant data forms.

- A GPS.

Implementation

The first phase is the preparation of the campaign, with training of people if needed, design the sampling scheme according to densities of cetaceans (if known) and habitats. Also, everything concerning authorization request and logistic should be launch largely before.

An acoustic acquisition chain is setup, comprising a tow cable into which is incorporated a linear array of two pairs of hydrophones, a deck cable that connects to the tow cable and carries signals to wherever the PAM station is set up. The electronic equipment at the PAM station provides power to the system, amplifies and digitises signals before feeding signals to one or more PCs that provide the user interface (software) and store the data. **If continuous acoustic detection is chosen, the vessel starts the transect with the acoustic acquisition chain in position. The start of the effort is when the acoustic detection of animals is launch.**

If **sampling procedure is used**, that means that regularly a listening period is implemented. For example, the standard is to listen for 2 minutes during each 15 minutes. Often, the speed of the boat is decreased at minimum in order to reduce engine noise and noise of the water flowing on the hydrophone.

Using hydrophone at sea is often linked to special authorizations to acquired.

Advantages

- This method is cost-effective, autonomous and it provides valuable information without disturbance to wildlife or their habitats.

- The detected radius can be very large for some species: most Mysticeti can be detected at tens or hundreds of kilometers. Depending on the equipment used, the ambient noise and the characteristic of the water for acoustic propagation, dolphins can be detected at distances up to 3 km in good conditions.

- The acoustic approach potentially detects the presence of a cetacean that is not visually observable because it is too far, it remains underwater, it moves at night or the weather conditions deteriorate. This method offers a valuable alternative for monitoring biodiversity when traditional (e.g. visual) surveys are impractical or impossible.

- Acoustic work can easily be done on a great type of vessels, from small boats or even opportunistic platforms to large vessel.

- This technique is not intrusive and the necessary equipment is not particularly expensive.

- This approach records sound for documentation or future analysis and it is easier to standardize and automate data collection.

- A key benefit of active acoustic methods lies in their fine spatial resolution and their ability to collect data on multiple species simultaneously and nearly continuously from a moving vessel.

- Acoustic data are largely independent of collection error and inter-observer bias.

- A mobile approach grants larger geographic coverage.

Limitations

- This method relies upon animals being vocal.

- Methods to relate sounds to abundance of animals are not well developed. In case of numerous animals, it is impossible to know which individual emits the sound and it is very difficult to know the number of animals in a group.

- Difficult identification for close species, mainly small dolphins (e.g. striped dolphin and common dolphin)

- Acoustic behaviour depends on the activity of a group, not necessarily the number of individuals, which can move without making any sound.

- Ambient noise and the noise generated by the research vessel can make the acoustic detection of an animal difficult. Detection probability is also a function of background noise, with acoustic interferences such as masking potentially species identification and group size estimation.

- Requires specialist data collection equipment.

- The volume of data typically generated by passive acoustic methods is enormous and requires significant investment in storage and after in post-processing.

- Small towed hydrophones are not suitable for the detection of low-frequency and infrasonic sounds simply because the vibrations and movements of hydrophones mask these sounds.

- Almost all hydrophones are sensitive to frequencies from a few hertz. This is why it is often necessary to use a high-pass filter to remove low-frequency noise.

3.3.2.2. Fix passive acoustic

Principle

One (or more) hydrophone(s) is installed in one (or more) fixed strategic sites, either on the ground, or on a boat or a floating platform. Opportunistic or non-dedicated platforms or stations can be used. Sound recording is done continuously or at a regular frequency (sampling). Positioning at least three hydrophones also allows triangulation to precisely locate the animal emitting the sounds. The more hydrophones, the larger the area covered. So, network of several hydrophones are necessary to increase the interest of such tool for monitoring the presence and indices of abundance of several species.

Human resources

At least one acoustician should build the acoustic acquisition chain. Then, depending on the situation (coastal or at sea), a ship with pilot should be needed and one diver will setup the system out at sea. The same people might be needed when the equipment has to be changed (batteries if any, hard drive when it is full,…).

Material needed

- A stereo hydrophone amplifier coupled to a transmission cable, a DAQ converter (digital and quantization of the signal), an hard drive to store data, a power source to power everything and finally a protection unit and fixations to install all equipment.

- A thermometer and a probe coupled to the sub-sea installation to enrich the data.

Implementation

The site is identified, the type of fixation is defined (depending on ground type, currents, etc) and the hydrophone system is installed. An existing underwater structure can be used, but caution should be made on the noise made by the structure, the more silent the better. Divers may install the acoustic system which will collect data for a predetermined period, mostly depending on capacity storage or power supply of the batteries. Then records (data) are being recovered for analysis. The system can stay for short, medium or long period. The recovering of the data and the changing of the batteries can sometimes be done without removing the whole system.

Using hydrophone at sea is often linked to special authorizations to acquired.

Advantages

- Passive hydroacoustic is ideal in long-term monitoring programs and can run on continuous 24-hour cycles, independently of weather conditions. By recording all animals moving close to a given listening station, it is possible to study temporal variations, ranging from the annual scale, to the monthly and daily scale.

- This technique is non-invasive and the cost of basic equipment is not very high.

- Acoustic data are largely independent of collection error and inter-observer bias.

- The system can be automated and requires no human presence on site. It is easier to standardize and automate data collection.

- Detection over 360° and in almost all weather and light conditions.

- If the installed system is permanent, detection and temporal coverage will work 100%.

- Depending on how the hydrophone is positioned, the material, the water characteristics of sound propagation and the ambient noise, the monitoring area for dolphins is about 3-6 km because there is no noise from the boat. Tracking sperm whales and the Mysticeti can be extended to tens of kilometers.

- The system can sample regularly or continuously areas that are difficult to access.

- Concerning the surface system on a floating platform:

- It can be self-contained with a power supply from solar panels or wind turbines.
- Data can be transmitted via VHF waves or Wi-Fi, allowing real-time application.
- Settings can be changed easily by easily accessible instruments (gain, filters, etc.).

- Concerning the system deployed on the sea bed:

- Discreet and less vulnerable to surface activities.

Limitations

- Detection probability and receiver performance are also a function of background noise, with acoustic interferences such as masking potentially hampering species identification and group size estimation

- This method relies upon animals being vocal.

- In this fixed method, the coverage is limited to the “immediate” vicinity of the system.

- Corrosion, fouling, and damage from currents, tides, storms, or fishery operations can all affect the longevity and efficiency of acoustic instruments.

- Methods to relate sounds to abundance of animals are not well developed. When animals are in a group, it becomes difficult to identify the individual that issued the sound and how many animals are present. There is a risk of multiple detection of the same group.

- Areas subject to strong tidal currents should be avoided due to noise or risk of damage to facilities (current, debris, etc.).

- Noise near the coast can mask the acoustic detection of an animal.

- Acoustic behavior depends on the activity of a group, not necessarily the number of individuals, which can move without making any sound.

- As part of a network of permanently installed hydrophones to detect all species, including those that emit very low or very high frequencies, the cost of the equipment required is very high.

- It is hard to differentiate between small dolphins species

- Concerning the surface system on a floating platform:

- Susceptible to all weather conditions on the surface ;
- Vulnerable to all activities taking place in the area (possibility of degradation or loss of the equipment) and preferably protected from free access of people.

- Concerning the system deployed on the sea bed:

- The power supply is complicated (cable? battery to change?) ;
- Need to dive in the site to change settings, difficult access to instruments ;
- What type of data transmission: by cable or storage?

3.3.3. Monitoring based on focal tracking of individuals

The previous methods described work more at a population level. Some specific monitoring focus on individuals. When the samples are numerous, they can give results at the population scales. Most of these methods are complementary to the previous ones, providing information to help to define ‘population’ for example, apart for photo-identification that can produce population estimates directly, through mark-recapture. Biopsy provide valuable data to the indicator 5.

3.3.3.1. Photo-Identification (or photo-ID)

Principle

Scientists use the photo-identification to distinguish cetaceans from each other, and recognize them. The technique relies on being able to obtain good quality photos of animals’ body parts that constitute unique recognizable markings during their whole life. The animals are photographed and catalogued individually based on natural markings criteria (e.g., pigmentation on the body, shape of the dorsal fin) and personal markings (scores, notches and scars) that identify them. A number of assumptions are made, particularly relating to recognizability, representativeness of sampling and capture probabilities that should be homogeneous. When an already identified individual is re-sighted, or photographically re-captured, this can provide a response to various issues, such as : population size, site fidelity, distribution, movements, social structure, etc. This means that there is a need for sorting, storing pictures and associated data within a catalogue which should be regularly updated.

Photo-identification is a good method to estimate population size (indicator 4) through mark-recapture models, and for specific areas that populations or part of populations occupy during one or more seasons of the year. It is also one of the methods to provide population parameters e.g. survival and calving rate.

The standard software program for mark-recapture analysis is program MARK (http://www.cnr.colostate.edu/~gwhite/mark/mark.htm), which includes a wide range of models to estimate population size, survival rates and allow to correct some of the bias against the assumptions.

Human resources

At least one trained observer/photograph will take pictures of the cetaceans and indicate to the pilot of the vessel how to move the vessel in order to ensure good photo-identification (speed, heading, position in comparison of the animals…). The post-treatment of pictures requests one skilled person at least, and is time-consuming, in order to get a final catalogue of photo-identified animals and the matrix of recaptures which is the base of any analysis.

Material needed

- A boat with a sufficiently low bridge over the water to take pictures at the correct angle.

- Observation forms and, ideally, a computer.

- A watch.

- A GPS.

- A camera with a lens (up to at least 200mm, ideally up to 300 or 400 mm). Digital cameras with high resolution (at least 6 megapixels) are highly recommended.

- a computer and a hard drive to store all the pictures and moreover the catalogue of photo-identified animals

Implementation

On the boat, researchers take pictures of natural markings on animals at certain angle and from certain parts of the body depending on the species (e.g. flanks for delphinids, tail for sperm whale) of all individuals encountered.

The analysis of the images is time-consuming and requires great concentration and attention to detail. Every individual is listed in a catalog of photo-identification, allowing comparisons. Scientist has to compare the photo of an individual with all the photos which are in his database and update regularly his existing catalogue and the matrix of re-capture. In an attempt to facilitate the process of matching, some software has been developed to make the comparison automaticicaly. The principle is that the software presents a number of candidates (possible matches) with a certain probability/similarity, which safes time to the researcher by not needing to go through the whole catalogue. Nevertheless, the researcher takes the final decision about a positive match.

Photography may require some specifics authorizations procedures as well as regional partnerships may require some agreements.

Advantages

- Relatively easy data collection protocol.

- Non-intrusive method of "marking" animals.

- A systematic sampling plan is not always necessary but is preferable.

- Standard and tested analysis methods exist, that provide reliable results as long as the hypotheses are tested or the bias are well estimated.

Limitations

- Only applicable for species with long-lasting identifiable natural marks.

- Natural marks must be unique, recognizable and not change.

- Heterogeneity of capture probability.

- The collected data is a photograph of a wild animal in motion; it is not easy to take a good quality photograph with targeted criteria without good relative experience.

- Required several captures. If there is not enough recaptures, analyses are difficult and sometimes give unreliable results.

- Require a large quantity of data and a long-term study and is time-consuming for the cataloging part.

- Difficulty of application in low-density areas.

- This method generates mark-recapture estimates of the total number of individuals in the study area. However, the total size of the population may be greater if all the animals in the population do not frequent the monitored area.

3.3.3.2. Telemetry

Principle

There are two types: satellite telemetry (Argos) and radio wave (VHF) telemetry. This technique consists in attaching a transmitter to an animal and following its movements remotely by satellite or via a receiver VHF or acoustics which can be installed aboard a ship or a plane.

Thanks to the beacons which transmit every hour/day their signals to the satellites, scientists acquire knowledge on the localization of the animal. These techniques allow to study animals in their world and to obtain information on feeding behaviour, distribution, reproduction area and migratory routes. These beacons also allow to record other data such as temperature, pressure, luminosity, swimming speed and sounds.

Information on the movements and distribution of individual animals can help to identify important habitats (feeding areas), migration routes and to define boundaries between populations. So, these data can provide complementary results to the indicator 3 at least and help to define the study area to monitor a population in the frame of the indicator 4.

Human resources

At least at sea, one person should have skills to attach/deploy the system on the animals. To detect the animal, and follow with VHF, at least 3 people are needed.

Material needed

- transmitters (Argos or VHF)

- small or relatively small boat (outboard or an average zodiac boat) with a sufficiently low bridge over the water to approach correctly the animal.

- beacon, crossbow or long pole

- In case of radio telemetry, a receiver VHF or acoustics to set up on a platform (vessel, aircraft) that follows the animal tagged.

Implementation

An animal will be detected and approached nearby, in order to attach (suction cup) or deployed the transmitter. Usually suction cups are pressed on the body using a pole, meaning to approach the animal to touch its body, whereas for Argos transmitters it is deployed in pulling on the animal with a crossbow a device with a clip that will be embedded in the subcutaneous fat of the animal.

For coastal species the approach can be made from a rubber boat directly, and for more pelagic species a large vessel can act as a base and a rubber boat can be towed and be used to approach the animals. For a device using VHF, the vessel will follow the animal at distance in order not to interfere with its behavior and also in order to recover the device when it will naturally get off the animal.

Because this method has a direct impact on cetaceans, it requires request of authorization prior to implementation.

Advantages

- These instruments allow to collect a lot of information not allowed by other methods (behavior, movements) and without human interference.

- This method allows to study movements of animals on a large distance, in isolated area and under the water surface.

For satellite telemetry:

- Operate on a very vast area and allows to study movements of animals on a large distance;
- Independent from weather conditions;
- Possibility to obtain additional information;
- No need of an observation platform following the animal at sea;
- Allows to know species presence in an unexplored area;
- Allows to obtain information summaries about the animal’s activities during long periods.

For radio telemetry:

- Relatively low-cost;
- Small-sized system and relatively non-invasive system;
- Operate on a wide area;
- Relatively independent from weather conditions.

Limitations

- This method is intrusive, either by its approach really nearly to touch the animal but also through the system to attach the device (mainly satellite transmitters) to animal body

- Information is obtained on few individuals and also depend on performances of equipment used, as well as the accessibility of mammals. A lot of individuals must be tagged to draw any general conclusions and this is often not possible

- The implementation of this method requires important logistical support because it requires an installation directly on the animal, which is a particularly difficult operation for rare and fast animals.

- This method is intrusive for animals, with infection risks.

- Only animals which can be correctly approached are equipped and required that the animal is at the surface for the data transmission

For satellite telemetry:

- Expensive method;

- Limited support of non-intrusive mechanism on animal and limited time-life.

For radio telemetry:

- Required to maintain a platform following the animal at close distance;

- limited autonomy;

3.3.3.3. *Biopsy*

Principle

This method consists in collecting on living animals at sea a fragment of skin and blubber. This can be done by throwing with a crossbow darts with tip, dart gun, riffle or even a pole with biopsy tip or skin swabbing when dealing with bowriding animals for example.

Such samples allow to gather information on biodemographic parameters (indicator 5):

- To determine the sex of the animal
- To determine the genetic specificity of individuals (fragment of DNA) of the same species. Based on that, analyses of kinship, matrilinear links, and social structure can be run.
- To obtain information on the reproductive status of individuals (e.g., pregnancy for females) based on the level of hormones.

Other information can be gain:

- on feeding level (isotope)
- on level of contamination in heavy metals and other pollutants (such as organochlorine contaminants)

Several parameters included in the indicator 5 can be obtained through the analysis of the skin and blubber collected with the biopsy method: sex ratio, pregnancy rates. Also, the genetic structure of the animals allows to better determine the limit of a “population”, or a sub-population, which helps to know when looking for the distribution or abundance of this population.

Human resources

At least one pilot, one shooter and it is highly recommended to have a photographer to be able to identify the animal sampled, which may provide the opportunity, for instance, of monitoring the healing process. A fourth person can take care of the samples when the biopsy has succeeded.

Material needed

- A small or relatively small boat (outboard or an average zodiac boat) with a sufficiently low bridge over the water to shoot at the correct angle.

- Crossbow or gun and bolts, darts with tip.

- Storage and cleaning material (products)

- Freezer or storage frozen.

Implementation

Animal targeted should be approached nearby. Biopsies are realized by means of an arrow (pulled by a crossbow or an airgun) which, pulled with some force, take a piece of skin and fall into the water where it is then recovered with the sample. In the same time, a photo allowing to identify animal is taken to obtain a complete documentation for each animal. It should be noticed that the material (skin and blubber) is right away stored following a strict protocol which can differ depending on the planned analyses (genetic, hormone, isotope): alcohol in one case, freezing in another.

As for photo-Identification, for coastal species the approach can be made from a rubber boat directly, and for more pelagic species a large vessel can act as a base and a rubber boat can be towed and be used to approach the animals whereas the large vessel stays away.

Because this method has a direct impact on cetaceans, it requires demands of previous authorization applications.

Advantages

- Give access to information very difficult to obtain in another way (genetic, hormones, isotope)

- Biopsy sampling tends to be relatively affordable and can be easily paired with additional methods to maximize data collection opportunities.

Limitations

- A strong disadvantage of biopsy is that it is invasive because the animal will be approached very near and the biopsy itself (i.e. results in physical lesions), which restricts sampling to the size and age classes (and species) that can be ethically targeted under existing permitting restrictions.

- The lifestyle of cetaceans, which spend only some fractions of their life on-surface limit strongly options to collect tissue from alive animals.

3.3.3.4. *Land based tracking*

Principle

This method consists in collecting data from a fixed point on the coast, following individuals crossing the area watched from the point of observation. Ideally, the point of observation must be high. Such tracking allows studying distribution, behavior, use of the habitat and movements of focal cetaceans, without impact of boat presence on the natural behavior of animals. This method is suited for the study of a coastal resident population or migrations close to the coast.

This method is most efficient for coastal population or resident groups. It can give results on distribution and habitat use, in link with indicator 3.

Human resources

At least 3 persons should be in charge of the observation and measures. One can make the measures of the group/animal followed, the second record notes, and the third one observes other part of the sea to detect other animals.

Material needed

- Binoculars or a telescope on a tripod.

- Observation form or Dictaphone.

- Watch or clock.

- Compass or angleboard and an instrument to measure the distance between the animal and the observation post (e.g., clinometer camera for photogrammetry, theodolites).

Implementation

One or more observers position themselves at a strategic point of view (headland, cliff, strait, entrance of a bay) and collects data on animals and weather. Observations can be made with naked eye or with binoculars or telescopes, but is dependent on a calm sea and on a good atmospheric visibility.

This type of monitoring does not require some special authorization procedures as long as the observation point is free of access.

Advantages

- Land-based methods are non-invasive, enabling the monitoring of marine mammals without risks of observer-induced disturbance.

- This is the least expensive techniques (no costs due to platform navigating at sea) used. It can therefore be implemented often and so allow a long-term monitoring.

- The land-based method can be easily standardized and realized all year round, according to observation conditions.

Limitations

- The field of study is limited to the area covered visually (naked eye or binoculars); the prospecting area is thus limited.

- Land-based methods are normally constrained to relatively conspicuous species that regularly come to the surface within sight of land.

- Investigations on fine-scale distribution are constrained by the difficulty in determining the precise geographical position of cetaceans. Theodolites are widely used in such studies, but there are limitations to their use. In particular, measurement readings can often be long and the collection is made on a center of gravity of a small group rather than on individuals. In addition, such groups can be spread over tens or hundreds of meters; a single position is rarely representative of all individuals.

3.4. Standard monitoring of strandings and by-catch animals

The monitoring of strandings and by-catch deal most of the time with dead animals.

A lot of data can be collected which will be used in the three indicators: as a first step, the collection of strandings and by-catch information aids the construction of a species list of cetaceans present in the area (or surroundings for strandings) and a rough measure of status and seasonal variation in abundance. Then the analysis of carcasses give a lot of information on demographic parameters.

3.4.1. Stranding

Principle

Stranding is a monitoring method that is continuous all year round, with qualified people ready to go on each stranding event of cetaceans when it occurs and is detected. Parameters of the animals are measured and biological samples are taken when possible and stored.

This method was the first one to be used by scientists as monitoring method, because strandings occur all the time and animals arrive on the coast, so they are easier to approach than living animals at sea.

Stranding of cetaceans represents an extremely precious scientific material for the knowledge of these species difficult to study in their natural environment. Study of carcasses, realization of autopsies and complementary analyses on biological samplings can supply information on the presence of a species, its distribution, demography of populations, feeding regime, health status of the animal (food, diseases, contamination), death causes, impact of anthropological threats (incidental catches, ship strike). These data will be used mainly for the indicator 5.

It is of crucial importance to fund this monitoring on long term and in a structured way. A network of referenced people localised all along the coast and working in the same manner, linked to a coordinator, is the base of an efficient monitoring network of strandings. An animation and steering committee would allow the network to function properly and guarantee the system's sustainability.

Human resources

People trained to do the measurements and take biological samples according to specific standard protocols, available to reach the stranded animals as soon as it is detected. Within the network there should be also veterinarians to examine carcasses, detect the causes of mortalities and place to store the biological samples (freezer).

Material needed

- Stranding forms
- Camera
- Tape measure
- Sampling kit (knife, shears, packaging materials)
- Refrigerated box and freezers network
- Dedicated dress, safety gloves, safety glasses
- Heavy equipment allowing to move carcass if necessary (bulldozers, rendering truck, car)

Implementation

When a cetacean stranding is reported, one or more person is on the scene to prevent the approach of people and animals to the carcass, and take measures and biological samples. This method requires a specific training for participants. A warning procedure must to be establish to be effective. A stranding network must be developed to be efficient and bring useful data.

Approaching and dealing with dead animals as well as protected species need special authorization.

Advantages

- Stranding bring even frequently information, even if these are often limited and non-predictable due to their nature.

- Availability of the whole body and organs for analyses and conservation (tissue bank).

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- Some species are known only by stranding and rarely observed at sea.

Limitations

- Not predictable and intervention must be realized on short time for sanitary reasons and for autopsy to be exploitable from a scientific point of view, so require to have an available person at the right time.

- Interventions on alive animals represent security and health risks for animals and rescuers. For animals, distress and stress engendered by stranding may cause unpredictable and dangerous behavior. Also, sanitary risks and disease transmission between rescuers and the animal are real.

3.4.2. By-catch

Principle

Marine mammals are frequently captured in fishing gear. "By-catch” means cetaceans accidentally captured by commercial fishing, sometimes but rarely by recreational fishing. Scientific observers can be embarked on board professional fishing ships, to observe captures and fishing conditions, and to take measures and biological samples.

Analysis of the measures and samples collected on carcasses provide a lot of information on demography (indicator 5): size of animals, age at maturity, rate of pregnancy, sex ratio…

Human resources

People trained to do the measurements and take biological samples of cetaceans according to specific standard protocols. Often, they might take other measures on other species when going on a commercial fishing vessel as observer. One person might go on one vessel for a period. This means that the most vessels to be monitored, the most people trained and authorized to board.

Material needed

- GPS, watch

- observation forms

- camera

- tape measure

- sampling kit (knife, shears, packaging materials)

- freezer

Implementation

One observer embarked on board of a professional fishing vessel. His work consists in collecting scientific data relative to the operation of fishing. He intervenes when a cetacean is captured to take data on the animal. If possible, he takes biological samples, stored them and go back at land with them.

To realize sampling on the individuals of marine mammals and bring them on land if useful and feasible, administrative authorization requests are necessary.

Advantages

- By-catch bring crucial biological information on "healthy" animals (compared to strandings who include sick animals), even if these are often limited and non-predictable due to their nature.

- All the animals by-caught might be "fresh" as they were alive few days before and biological samples might be taken from all of them, insuring availability of good quality samples for analyses.

- An observer aboard a fishing vessel will bring data on species and number of animals that are by-caught, enabling to assess the impact of this threat for cetaceans (provide complementary information for indicator 3 and 4).

Limitations

- The event of by-catch is rarely predictable, there might be no by-catch

- Difficulty in going aboard fishing vessel sometimes, because of willingness of fishing captains, size of the vessel or authorization,

- Difficulty in doing the measurements and taking biological samples in some small sized fishing vessel, and also in storing samples in a freezer.

- Intervention on a carcasse in a moving vessel represents security risks for people. Also, sanitary risks and disease transmission between people and the animal are real.

3.5. Emerging Monitoring technologies

As technologies are improving fast, new studies using them are launch. As these are relatively recent, case by case tested and relying upon technology's capacities (namely pictures resolution, autonomy of AUV, artificial intelligence software to analyses thousands of images, etc.) no standard method is yet approved or define. But as this field is of growing interest and development, and as these technologies may be use within the standard methods already presented in terms of improvements or adding values, these technologies will be shortly presented in this document.

3.5.1. Unmanned underwater and aerial vehicles

3.5.1.1. Sampling from Drone (pictures, blow…)

Advances in aerial drone technology offer new opportunities for studying cetaceans remotely and noninvasively. These instruments are light-weight, portable platforms piloted remotely from the ground/deck of a vessel, and allowing surveys of remote, hard-to-reach areas within small time windows.

Drones or Unmanned Aerial Vehicles (UAVs) can be used to take pictures or videos by applying the line transects method (visual), to answer abundance and distribution questions. As survey by aircraft, the protocol consists to program to follow a flight plan defined and surveyed along pre-determined transects based on GPS waypoints to form a full coverage survey grid. The drone takes a collection of images with an overlap in coverage of the survey area, and records flight information such as GPS coordinates and altitude in the EXIF header of each image file.

UAVs are a promising tool for animal surveys. Indeed, this technology has many advantages:

- potential for carrying out relatively large-scale aerial image-based surveys at often a fraction of the cost of manned aerial surveys, and without many of the safety issues associated with manned aircraft ;

- low cost of UAV systems compared to manned aircraft may also allow greater flexibility in survey design, for instance by flying two or more platforms at specific time lags rather than employing the circle-back maneuver ;
- ability to repeatedly collect high-resolution aerial imagery, with extremely low disturbance to animals ;
- possibility to be used in areas where manned aerial operations are difficult and dangerous, and allows to survey sites with no airfields ;
- may eliminate observer bias in the data collection phase ;
- less subject to flight restrictions due to weather conditions ;
- results are easily replicated and have minimal impact on the surrounding environment.

However, this technology has some limits :

- the longer manual data post-processing times still pose some challenges (in terms of efficiency and costs) ;
- environmental and survey-related variables, such as light conditions and wind, can affect detectability. Several studies are in progress to quantifying detectability and certainty in animal detections/identification using UAV technology ;
- the majority of available UAVs is only useable over limited ranges (i.e. within line-of-sight), at slow speeds, and under small payloads ;
- *stringent and country-specific civil aviation regulations and complex permitting processes can limit their adoption for scientific applications ;*
- the covered surface is still lower than the one from a plane ;
- impossibility to fly in high winds (wind speed must be less than 25 knots on the ground);
- requirement to stay within line-of-sight and 0.8 km of observer, and requires costly vessel for use as a transport .

A drone can be also used as tool to approach an animal realized from a boat. It can allow to study behavior by achieving better visibility or to take a sample such as in the blow of a whale. This system allows to non-invasively collect mucus microbiota samples safely and reliably, by minimizing external contamination such as air and seawater from outside the blowhole. This type of samples is used for hormonal analysis for example and can help for the indicator 5.

3.5.1.2. *Marine AUVs and glider*

An AUV is a marine craft pre-programmed to conduct underwater missions without constant supervision or monitoring by a human operator. They allow observations of species in their natural environment, with highly accurate vertical and horizontal geo-positioning and the ability to instantly react to the observed environment.

Ocean gliders are autonomous winged underwater vehicle that collects ocean data using buoyancy-based propulsion and can remain at sea for weeks to months at a time surveying over spatial scales from ones to hundreds of kilometers. Modern gliders can be fitted with cameras, mobile tracking systems, or acoustic loggers/echosounders. Some robots automatically detect those sounds, identify the species based on characteristics of the sounds, and report which species have been heard to scientists on shore via satellite in near real time.

Robots are powerful tools for accessing environments too dangerous or too remote for human exploration. They can complement conventional forms of sampling by providing long-term, fine-resolution coverage of areas that are impractical or too expensive to survey, without constraint from weather conditions or sea states.

Some instruments can remain unattended for several weeks to months, offering an unsurpassed level of autonomy.

Their biggest drawbacks are their high costs, slow speeds, and limited dive times. Furthermore, their energy storage and power consumption are some limits.

AUVs and ocean gliders are valuable for generating long-term datasets in remote locations but can be challenging to deploy and recover.

Launching an AUVs or glider within the sea may be constrained by some authorizations.

3.5.2. Pictures and video

Digital cameras delivering stills and video feeds can be used as a **support to observers** in order to gain some precision if needed. For example, they can be used during a sighting to precise group size count or identification of species. Conducted in a more continuous way, they may help in enhancing encounter rates, although usually within a narrower search swath located immediately beneath the plane. These technologies are helpful in being used in parallel, to combine the advantages of human observations for scanning larger regions with the advantages of later re-analysis and reassessment of images and videos.

Several studies are in progress to test if those technologies alone could be used as monitoring methods. Tests are in progress to allow an automatic detection and determination of cetaceans but methods are not yet operational. Aerial videography benefits from standardized methodologies that can be replicated, but is time-consuming and very costly, because the determination of cetaceans has to be done by an operator.

Taking pictures or video may be constrained by some authorizations.

4. Conclusion

Monitoring cetaceans is a hard task, based on the fact that they are highly mobile and spread in vast areas. Methods have been developed to collect data to follow the evolution, mainly of their distribution, their numbers and their demographic characteristics. Monitoring such parameters imply a lot of knowledge, skills and ressources. Each method has its advantages and disadvantages, and approaches may frequently complement one another in providing a more complete picture of the status and distribution of a particular cetacean species.

A least a strandings monitoring should be organized, with a strong network, everywhere for baseline data on cetaceans (distribution, presence, indices of abundance, genetic analysis). Then a first visual and acoustic survey should be organized over large scale for a knowledge about the global context, which could be repeated regularly several years later (6 to 10). Ferries and oceanographic vessels should be used as non-dedicated platforms if they cover an area on a regularly basis which can be important for cetaceans. Then more focused monitoring program covering smaller but representative or important areas should be launch on a yearly basis, including visual and acoustic with some biopsy and photo-ID.

Before embarking upon a monitoring programme, it is prudent to determine precisely what information can be gained and what limitations exist. A lot of practical and operational adaptation can be found on a case basis. A lot of monitoring programmes already exist, being a source of advises that should be ask for in order to gain at quality, logistical and cost levels.

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B. Guidelines for monitoring Mediterranean Monk seal

Table of Contents

I. Introduction

1. Background

In 2008, the Contracting Parties to the Barcelona Convention - namely 21 Mediterranean countries and the European Union (EU) – decided to apply the ecosystem approach (EcAp) to the management of human activities that may affect the Mediterranean marine and coastal environment for the promotion of sustainable development (UNEP/MAP, 2007). It is an ecological strategy for the integrated management of land, water and living resources that promotes conservation and sustainable use in an equitable way, with the aim to ensure that human use of ecosystems is kept within the limits of capacity of ecosystem. The ultimate objective of this approach is to achieve the Good Environmental Status (GES) through informed management decisions, based on integrated quantitative assessment and monitoring of the Marine and Coastal Environment of the Mediterranean.

In 2016, the Contracting Parties also agreed to design an Integrated Monitoring and Assessment Programme (IMAP) with a list of regionally agreed good environmental status descriptions, common indicators and targets, with principles and clear timeline for its implementation according to the 6 year-EcAp cycles structure. Building and implementation of a regional monitoring system is the main goal of IMAP to gather reliable and up-to-date data and information on the marine and coastal Mediterranean environment. By adopting IMAP, Mediterranean countries committed to monitor and report on Ecological Objectives (EOs) and their related common indicators (CIs), in synergy with the EU Marine Strategy Framework Directive (MSFD), covering three components: i) biodiversity and non-indigenous species; ii) pollution and marine litter; and iii) coast and hydrography.

One of eleven ecological objectives is “Biodiversity is maintained or enhanced” (EO1). The term ‘maintained’ is key to the quantification of GES for EO1. This condition has three determining factors:

4. no further loss of the diversity within species, between species and of habitats/communities and ecosystems at ecologically relevant scales;
5. any deteriorated attributes of biological diversity are restored to and maintained at or above target levels, where intrinsic conditions allow;
6. where the use of the marine environment is sustainable.

Among five common indicators related to biodiversity (EO1) fixed by IMAP, three are about marine mammals including the Mediterranean monk seal:

- Common indicator 3: Species distributional range;
- Common indicator 4: Population abundance of selected species;
- Common indicator 5: Population demographic characteristics (e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates)

2. Purpose and Aims

As top predators in the Mediterranean Sea, the monk seals are an important element of marine biodiversity. Their abundance and distribution are known to respond to various natural and anthropogenic drivers. Role of long-term monitoring programmes in assessing population states are widely recognized and several programmes covering the North-East Atlantic marine environment including plankton, fish, seabirds and marine mammals already in operation. Monitoring efforts of Mediterranean monk seals are regional due to their scattered distribution range. The largest subpopulation inhabits the eastern Mediterranean Sea in Greece and Turkey. The second largest aggregation located at Cabo Blanco. The third subpopulation inhabit the archipelago of Madeira and the small unknown number of seals might inhabit at the eastern Morocco therefore every working group has a different monitoring strategy regarding their regional differences.

The aim of this document is to provide guidance to monitor Mediterranean monk seal in relation to the IMAP common indicators, i.e distribution, abundance and population demographic characteristics (i.e.

Body size or age class structure, sex ratio, fecundity rates, survival/mortality rates) at the Mediterranean and national scale.

These monitoring guidelines are for the surveys to be conducted in the areas where the Mediterranean monk seal populations actively occur/inhabit.

3. Common Indicators related to Marine Mammals including the Mediterranean monk seal

A common indicator is built in the context of the Barcelona Convention and it "summarizes data into a simple, standardized, and communicable figure and is ideally applicable in the whole Mediterranean basin, or at least on the level of sub-regions, and is monitored by all Contracting Parties. A common indicator is able to give an indication of the degree of threat or change in the marine ecosystem and can deliver valuable information to decision makers (IMAP, 2017)".

Among five common indicators related to biodiversity (EO1) fixed by IMAP, three are about marine mammals:

- Common Indicator 3 - Species distributional range:
  This indicator is aimed at providing information about the geographical area in which marine mammal species occur. It is intended to determine the species range of cetaceans and seals that are present in Mediterranean waters, with a special focus on the species selected by the Parties. The main outputs of the monitoring under this indicator will be maps of species presence, distribution and occurrence.
- Common Indicator 4 - Population abundance of selected species:
  This indicator refers to the total number of individuals belonging to a population in a specified area in a given timeframe. Methods for estimating density and abundance are generally species-specific and ecological characteristics of a target species should be considered carefully when planning a research campaign. In this document, target species refers to the Mediterranean monk seal.
- Common Indicator 5 - Population demographic characteristics (e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates):
  This indicator aims to provide information about demographic parameters as the age structure, age at sexual maturity, sex ratio and rates of birth (fecundity) and of death (mortality). These data are particularly difficult to obtain for marine mammals. Monitoring effort should be directed to collect long-term data series covering the various life stages of the selected species. This would involve the participation of several teams using standard methodologies and covering sites of particular importance for the key life stages of the target species.

6. Monitoring methods

6.1. Monitoring strategy

Due to the very critical status of the Mediterranean monk seal, any type of monitoring activity of the species should be conducted under the supervision of the national authorized legislative bodies.

The Mediterranean monk seals spent most of their time in the water, however, monitoring them in the aquatic environment is a challenging job and provide little information on the population. On the other hand, they marine caves while haul out to rest and breed and this period is the best option to collect data on the species. The most suitable method to monitor the Mediterranean monk seals in their cave is to use non-deterring camera traps in order to minimize disturbance while monitoring.

2.1.1. Time, Place and period

In general, monitoring should be performed all year round. However, if there is any restriction to due to season, location of cave, camera trap availability, the effort should be concentrated in monitoring only the breeding caves during the breeding season, which almost exclusively takes place between August to December in the Eastern Mediterranean Sea. There are, however, not enough scientific evidences to propose that the breeding of the Mediterranean monk seals is strictly seasonal and could therefore show a regional difference elsewhere.

2.1.2. Equipment

The following is the basic equipment needed for cave monitoring

- A boat preferably and inflatable one is essential to reach the seal habitats
- Camera trap with PIR-based motion detector
- Silicone sealant to be applied to the camera traps for extra protection against excess humidity
- Waterproof dry bag and container to carry the camera traps and other electronic equipment
- Flash memory card (16 GB or higher)
- Personal Free diving equipment (ABC equipment)
- Underwater torch
- Hand hold GPS to record the position of the caves
- Photo-trap cave-wall mounter (preferably made of chromium, custom-built)
- Protective equipment as required (such as (life vest, helmet, etc.)

For land-based surveys a photo camera with telephoto-lens (200-400 mm) high magnification binocular may also be used

2.1.3. Maintenance of Equipment

The most important equipment of monk seal surveys is camera trap. It is not waterproof but is weather resistant. As camera-traps are deployed for long times in a cave environment that is extremely humid, additional protection should be applied such as sealing the joints of the body with silicon sealant. Placing a small umbrella like protection may be considered to prevent equipment from dripping water. Batteries of GPS and underwater torches are checked before every survey. Setup of camera-traps should also be set considering the status of the environment in which the camera traps are to be deployed. Metal (containing) equipment should be lubricated against corrosion after every use. After the camera trap recovery, memory cards and batteries should be removed from the traps, and are cleaned to remove sea salt.

2.2. Monitoring methods

2.2.1. Primary monitoring methods

2.2.1.1. Cave survey and monitoring

As mentioned before, the best monitoring method of the Mediterranean monk seals is to observe them in their haul out habitats (i.e. marine caves). Within this scope, cave surveys should be conducted to identify caves that are suitable for monk seal use. Then, the caves that are actively used by monk seals are monitored by camera-traps in order to minimize disturbance while monitoring the population.

2.2.1.2. Surveys to explore resting/breeding habitats

i. In areas not surveyed before

Surveys should be conducted in areas not investigated before to explore caves which meet the requirements and descriptions of a Mediterranean monk seal cave (IUCN/UNEP, 1998). Active surveys should be carried out on coasts where the geography is suitable for cave formation. For that respect, karst steep topographies are of great importance. The surveys should be done using a boat manned preferably by four people; two swimming along the coast of interest in search of caves; one recording the data and one steering the boat. The monk seal cave might may have underwater entrance with very narrow passage and a long corridor, so it is not always easily recognizable from surface. The large and narrow openings, crevices and holes between the rocks should therefore be checked carefully. When an entrance is found, a team member should enter the cave with necessary precautions taken in order not to disturb the animals. Caves with underwater entrances should always be investigated by free diving. Noisy equipment, such as scuba diving equipment are not recommended for cave investigations as the disturbance created by the bubbles can deter the seals. If the entrance of a cave is too long to be enter on apnea, SCUBA equipment may be used only for exploration.

ii. In areas surveyed before

If the area has already been surveyed before and an available information about the marine caves are available to identify the caves to monitor, the procedures explained in the section above can be neglected. However, in any case, surveys are recommended to cover the whole area at least once as Mediterranean monk seals can also use protected and deep crevices for resting.

2.2.1.2.1. Cave Inventory

Information of newly explored caves should be recorded in both a field survey (Annex 1) and a cave inventory protocol sheets (Annex 2). The cave inventory protocol includes the coordinates of the cave and various characteristics of the cave related to the Mediterranean monk seal monitoring including number of entrances, resting platforms, air chambers, its photograph, total length, its sketch where possible etc. Each cave should also be classified according to the categories described by Gucu et al. (2004).

2.2.1.2.2. Selection of caves for monitoring

The height of the ceiling and width of the inner space of actively used caves are taken into consideration to evaluate the risk that the camera could be exposed to strong waves while selecting a cave for monitoring. In order to prevent loss of camera-traps, the caves that has ceiling lower than the maximum wave height are not used for monitoring. Combination of various factors such as the season, accessibility, cave type (potential, active or breeding) and cave characteristics, number of available camera traps is effective of selection of caves for monitoring. However, if year-round monitoring is not possible, then emphasis should be given to the breeding caves during the breeding season, as fecundity is utmost important population parameter to be monitored.

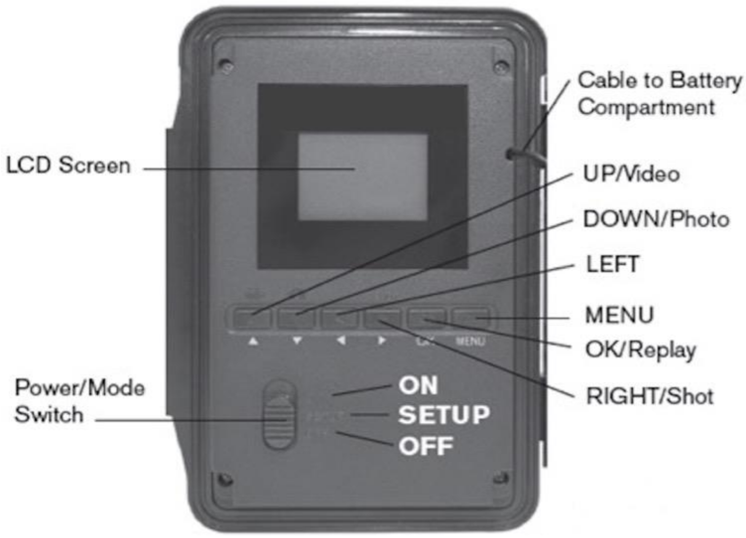
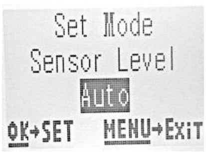
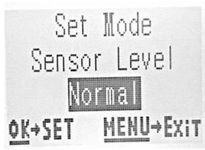
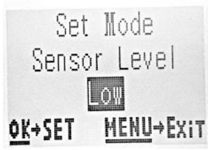
2.2.1.2.3. Camera trap set up, deployment, and recovery

Commercially available camera traps have photograph, video and hybrid modes. The hybrid mode allows both still photos and videos to be captured at each trigger so may be good for data collection on behaviour. Camera image size should be in the highest resolution as high-quality photographs are needed for the photo-identification analyses. The length of the video captures should be set considering the duration of deployment, battery life and the size of the memory card.

Data and time stamp of the camera-trap is crucially important for the data stored in the memory cards. The built-in clock should be set with care and stamp mode should be set on “ON”. Some camera-traps has built-in temperature and moon stamps, which may be useful to have more information about the in-cave seal behaviour.

Most commercial camera-traps will take a photo (or record a video clip) automatically at your choice of time intervals to prevent the card from filling up with too many redundant images and to prolong battery life. The interval between two consecutive activations may be set at 20 minutes and longer in order to minimize disturbance (Gucu 2009). Sensor setting is set to auto or to normal/medium if the auto option is not available as in the case of some models. If the other fauna (bats, rats, etc.) is observed in the cave, a low sensitivity of sensor settings may be used to avoid unnecessary activation of the camera trap by this fauna (Table 1).

Table 1. The basic camera set-up for monk seal cave survey/monitoring

Basic camera trap set-up for monk seal cave survey/monitoring	
Front view	**Back view**
	
Settings	**LCD screen view**
Camara Mode	 
Camera image size	
Video length if hybrid mode is set	
Event interval	
Sensor level	   or

Location of the camera-traps is determined in order to get appropriate photos that cover the right location where the animal hauls out most of its time in the cave. The number of traps used in a cave changes based on size and morphology of the cave. The caves with wide inner space where the haul out platforms are larger than the camera view angle is monitored with sufficient number of camera traps.

Photo-trap cave wall mounter is placed to the suitable location by nailing its legs. When the suitability of location is assured, it is permanently fixed by covering the legs with white cement. After drying of cement, camera trap is fixed to the mobile arm of the wall mounter by using screws. At last, tilt angle of the trap is checked, the paper cover over the PIR sensor is removed and the trap get activated. The camera trap is strengthened with plastic cable ties.

Depending on combination of various factors such as the season, accessibility, cave type (potential, active or breeding) and cave characteristics, camera traps are left in caves for one to the maximum of three months. During recovery, camera trap used is usually replaced with a new one, as the camera trap used is usually worn out due to the conditions in the cave. However, the flash card is replaced only if there is no spare camera-trap available and previous one is going to be kept in the cave for the next survey.

2.2.2. Secondary monitoring methods

The methods below are used in the Mediterranean monk seal monitoring, but the output is usually very limited. So, these methods are considered as complementary to the primary monitoring methods.

2.2.2.1. Land based survey

Land based survey is conducted by a team of two observers during daytime at a high point on land where presence of the monk seal is confirmed or previously reported. During the observations, information is collected on date and start and end times of observation, name and coordinates of observation point, weather conditions (taken at hourly intervals or when it changes), time of seal sighting, seal morphology and behaviour. Photos/videos are taken when possible. Survey lasts over 1 hour and is stopped if a seal does not appear after 2 hours of observation or, when the sighted seal disappeared from sight. As well as during cave surveys and monitoring, weather conditions (sea state, wind force and direction, and visibility) are also factors limiting the land-based surveys.

2.2.2.2. Opportunistic monitoring

i. Dedicated observers on opportunistic platform (i.e oceanographic vessel)

Surveys are performed by dedicated observers during daytime while the vessel is in transit. An observer is placed on the bridge of the research vessel, searches for the presence of the monk seal using both naked eye and binoculars. During the observations, information is collected on date and start and end times and coordinates of observation, weather conditions (taken at hourly intervals or when it changes), time of seal sighting, number of seals, morphology and behavior. Photos/videos are taken when possible. These observations are carried out when the research vessel is cruising at speeds not greater than 12 knots and weather conditions are relatively fair.

ii. Stranding

Information on stranded animal is recorded including the ID number, observation date, stranding location, latitude and longitude coordinates, length and weight of the animal (where possible to measure), age class, sex, stranding condition (live or dead), and other observational comments, including evidence of injury or human interaction. Photos/videos are taken where possible. Morphological features are mapped to a seal identification sheet. Data on stranding contributes the mortality rate estimations while evaluation demographic structure of the population.

2.2.3. Synthesis tables

Table 2. A synthesis table listing the different monitoring methods that can be used to monitor each common indicator.

Related to common indicators				
Monitoring methods	**CI 3** Species distributional range	**CI 4** Population abundance	**CI 5** Population demographic characteristics	**What to survey/monitor**
Surveys to explore resting/breeding habitats	x	x	x	• Seal presence/absence • Seal habitats • Seal habitat use • Basic demographic
Cave monitoring	x	x	x	• Basic demographic structure, parameters and trends • Seal habitat use • Seal behaviour Individual identification • Monitoring the habitats • Low cost • Can be used for public awareness
Land based surveys	0	0	x	• Seal presence/absence • Seal habitats • Seal habitat use • Behaviour
Dedicated observers on opportunistic platform (i.e. a research vessel)	x	x	x	• Seal presence/absence • Seal habitats
Stranding	x	x	x	• Input to basic demographic structure (specifically mortality rates)

Table 3. A synthesis table listing the different data analyses methods that can be used for each common indicator. X: the method is relevant ; 0: the method is not relevant

Data analyses methods/ Related to indicators	**CI 3 Species distributional range**	**CI 4 Population abundance**	**CI 5 Population demographic characteristics**
Photo-identification	x	x	x
Demographic analyses	0	x	x
Population Viability analyses	0	x	x
Mark-recapture analyses	0	x	0

Table 4. Synthesis table listing the equipment for the different research methods. X represents the equipment is used, 0 respresents the equipment that is not used

Equipment	Primary monitoring methods: Surveys to explore resting/breeding habitats	Primary monitoring methods: Cave monitoring	Secondary monitoring methods: Land based survey	Secondary monitoring methods: Opportunistic monitoring (from a vessel)	Secondary monitoring methods: Opportunistic monitoring (stranding)
Research vessel/ Inflatable boat	X	X	0	0	0
GPS	X	X	X	X	X
Photo/video camera	X	X	X	X	0
Underwater torch	X	X	0	0	0
Personal free diving equipment (mask, snorkel and fins) (ABC equipment)	X	X	0	0	0
Camera trap with PIR-based motion detector	X	X	0	0	0
Flash memory card	X	X	0	0	0
Photo-trap cave-wall mounter (chromium, custom-built)	X	X	0	0	0
Silicone sealant	X	X	0	0	0
Waterproof dry bag and container	X	X	0	0	0
Life vest	X	X	0	0	0
Various tools (such as plastic cable tie, nails, pliers)	X	X	0	0	0
Binoculars	0	0	X	X	0

Table 5. Synthesis table listing the equipment for the different monitoring methods.

Monitoring methodology	Advantage	Disadvantage
Surveys to explore resting/breeding habitats	▪ Updating/Identification of habitats	▪ High cost and logistic challenges

	▪ Updating/recording of habitat use	
Cave monitoring (with camera traps)	▪ Recording of basic demographic; structure, parameters and trends ▪ Recording of natural behaviour individual identification ▪ No/minimal disturbance ▪ Monitoring the habitats ▪ Low cost ▪ Can be used for public awareness	▪ Equipment is prone to water and damage ▪ Medium quality population estimates
Land based surveys	▪ Updating/Identification of habitats ▪ Updating/recording of habitat use ▪ Input to basic demographic structure ▪ Low cost and challenges	▪ Poor individual identification ▪ Low quality of population estimates
Dedicated observers on opportunistic platform (i.e. a research vessel)	▪ Updating/Identification of habitats ▪ Updating/recording of habitat use ▪ Input to basic demographic structure	▪ Poor individual identification ▪ Low quality of population estimates
Stranding	▪ Input to basic demographic structure (specifically mortality rates)	▪ Poor individual identification

3. Data analyses

3.1. Photo-Identification

Estimation of the population size of the Mediterranean Monk seals has a critical importance to assess status of the species. However, it is very challenging job considering their small numbers and isolated nature, therefore, methods used in cetacean studies such as tagging or observation from boats are not applicable for this species. Photo-ID on the other hand is another commonly used method on numerous species which is a practical alternative for monk seal studies.

The Mediterranean monk seal has distinguishable unique pelage patterns, scars, natural marks, that can be identified through high-resolution photographs and video footages taken by camera-traps. Pelage colour is not used to identify seals as it is dark and shiny when the seal just hauls out and gradually turns light grey as the animal get dried during resting. Obtained photographs are sorted by date and time to be able to identify seals photographed at the same time. Captured images are controlled and photographed seals are grouped regarding their sex and the morphological categories based on Samarach and Gonzalez (2000), Dendrinos et al. (1999), Ok (2006). The details of the morphological categories are given below in section 3.2.1. Morphological features mapped to a seal identification sheet (Annex 1). These sheets include dorsal, ventral, lateral drawings of the seals which can be full-filled manually. Finally, the sheets compiled in an identification catalogue that involves basic characteristics of the identified individuals such as sex, name, morphological stage, date of the first sight and habitat information.

3.2. Demographic structure

The demographic structure of the population is explored by using the approaches explained below.

3.2.1. Minimum estimated age

The minimum ages of the individuals are estimated according to the method given by Gucu et al (2004)

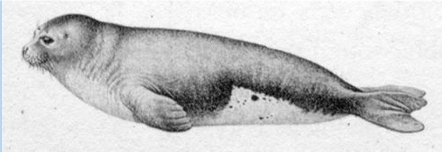
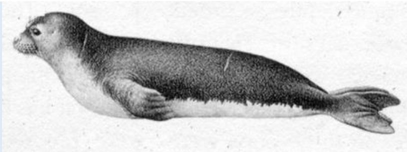
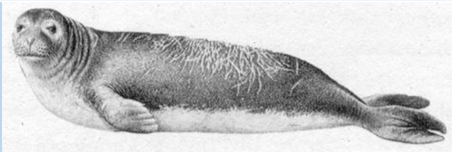
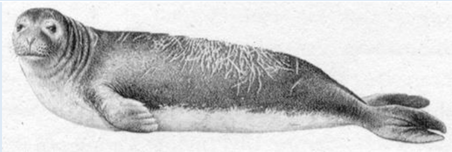
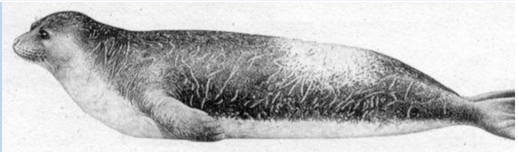
Estimated minimum age in years; Aest= (P-D)/365+X where

D: Date of the first sight.
P: Days transpired since the first sighting
X: the age of the individuals at the first sighting.

In order to estimate minimum age of an individual in years, the age of the individuals at the first sighting (X) is estimated by choosing one of the morphological categories described in Table 6.

Table 6. Modified morphological categories of the Mediterranean monk seal (taken from Ok, 2006).

Stage	Characteristics of the category	Period (years)	Photo/illustration Photos taken from Dendrinos et al. 1999 Illustrations taken from Samaranch and Gonzales, 2000
1	skinny (pup-premolted; pms)	0.00-0.03	
2	fat (pup-premolted; pmf)	0.03-0.08	
3	pwm moulting (pup-preweaned; pwm)	0.08-0.14	
4	pup-preweaned (pw)	0.14-0.33	

5	youngster- weaned (y)	0.33-2.50	
6	subadult (sa)	2.50-6.00	
7	adult female young (afy)	6.00-7.00	
8	adult male young (amy)	7.00-8.00	
9	adult female elder (afe)	8.00-20.00	
10	adult male elder (ame)	9.00-20.00	
11	senesce female (sf)	20.00- -	Not available

3.2.2. Fecundity

Fecundity of the population is calculated using the formula formed by Akçakaya et al. (1999)

Ft = Pt+1/At
Ft: Fecundity at time t.
Pt+1: Number of pups born at time t+1.
At: Number of parents at time t.

3.2.3. Annual birth rate

Annual birth rate of the population is calculated according to Gazo et al. (1999)

ABRt = Pt/ AFt
ABRt = Annual birth rate at time t
Pt = Number of pups born at time t
AFt = Number of sexually mature females (categories starting from 7 in Table 2) at time t

3.2.4. Survival and Mortality rates

Number of individuals and deaths (mainly stranded animals) are recorded for each year and used to calculate the annual mortality rate and subtract from one to obtain overall survival rate to the next year. Following formula of Akçakaya et al. (1999) summarizes the calculation:

$S_t = 1 - (D_{t+1} / N_t)$
S_t: Survival of the individuals at time t.
N_t: Number of individuals at time t.
D_{t+1}: Number of deaths at time t+1.

3.3. Additional Advanced methods

3.3.1. Population Viability Analysis

Population viability analysis is used to explore current and future status of the Mediterranean monk seals including the threats faced by species, risk of their extinction or decline, and their chances for recovery, based on species-specific data as described by Akçakaya et al. (1999). Various types of population models can employ depending on the structure of the population. A stage-structured stochastic population model is used as its groups individuals in a population according to their age or morphological characteristics, allowing vital rates (survival and fecundity) by age or stage-class to be integrated in the model (Akçakaya 2000). Model results are summarized in terms of population trajectories and risks of decline within different time durations and different parameters.

3.3.2. Mark-recapture Analyses

Data derived from photo-Identification is exploited in mark-recapture analyses. In this approach, re-sighting events of seals with distinctive markings are used to study the movement patterns, site fidelity, and population size (Karlsson, Hiby, Lundberg, 2005). More specifically, the marking recapturing index (Lancia et al., 1994) is used considering 2-sample closed population model of Lincoln-Petersen (Lincoln 1930). The first step is to capture and mark a sample of individuals. Marking methods depend on the species. In monk seals, identified individuals are assumed as marked individuals. The assumption behind mark-recapture methods is that the proportion of individuals identified in first control recaptured in the following period represents the proportion of identified individuals in the population as a whole.

4. Quality control

All the survey protocols filled are cross-checked between at least two members of the survey team. Photographs taken by camera-traps are scored by different researchers taking into account various factors such as image resolution, level of distinctiveness, visibility of natural marks. In order to test the accuracy of the photo-identification, the same set of photographs are assessed by different researchers. Each national monitoring group has its own quality control protocols. Although especially photo-identification methods used are similar, the selection, scoring, and matching of images are varied greatly amongst research groups. Therefore, it is recommended that a common protocol in quality control should be developed between the contracting parties.

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Annex 1: Field survey protocol

Date:			Time start:					Time Stop:
Team:					Survey type:			
Weather Conditions								
Wind speed:		Calm		Medium		Strong		
Cloudiness:	Bright		Partl	Cloud		Rainy		
Sea Condition								
Wave direction:								
Wave strength:	Cal	Mo	Ro	Sto	Sw			
Turbidity:	Clear		Blui	Green		Brow		
Tide		Up tide		Normal		Low tide		
The Coastal	From:		To:		**GPS file:**			
Caves Discovered								

Cave	L	L	Remark

Other survey remarks			
Event	Time	**Lat**	**Lon**

Annex 2: Cave inventory sheet

Cave code	**Cave name**	**Discovered by**
Cave Info		
Latitude	Longitude	Photo frame
Total length in meters (opening to far end)		
Number of seal (s) :	Sighting Code :	Odor :
Number of chambers	With air:	Without air:

Cave entrance information

Entrance #	Surface	Underw	Land	Depth	Height	Width	Direction

Platform information

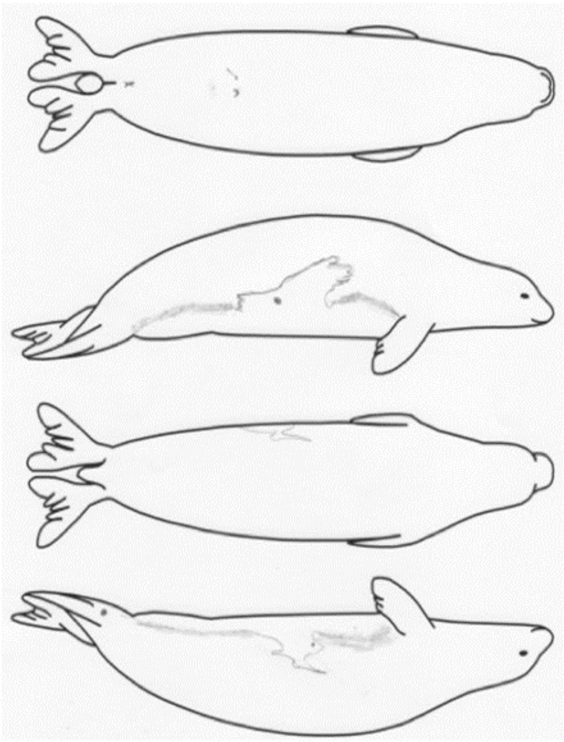
Platform	Positio	Length	Width	Texture	Suitabil	Feces	Fur	Track

Seal Evidence

Platform	Depression	Track	Fur	Feces	Other

Sketch of the cave

Annex 3: identification sheet



Code	: Y1
Sex	: Female (Youngster)
Sighted in	: Zafer Burnu
Cave(s) used	: Z1
Number of photos	: 20
Identification	: Ventral discoloration

C. Guidelines for monitoring sea birds in the Mediterranean

Table of Contents

Executive Summary

Conservation and wise use of marine ecosystems requires managing human activities. Sound scientific knowledge is needed to allow for adequate measures to be put in place. Monitoring and assessment of biological populations, and of the ecological conditions on which they depend, becomes essential to achieve the conservation objectives.

In the Mediterranean region, the UN Environment/MAP Barcelona Convention ***Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria*** **(IMAP)** defines the roadmap to deliver the implementation of the Ecosystem Approach Process (EcAp process), between 2016 and 2021, to assess the status of the Mediterranean Sea and coast, as a basis for further and/or strengthened measures.

In relation to seabirds, IMAP proposes to monitor and assess the following common indicators (CIs): **CI 3: Species distributional range** (EO1); **CI 4: Population abundance of selected species** (EO1); **CI 5: Population demographic characteristics** (EO1, e.g. body size, age class structure, sex ratio, fecundity rates, survival/mortality rates). IMAP recommends monitoring and assessing those common indicators for a selection of representative species, 11 in total, organised into 5 functional groups.

Functional groups aim to combine information on different species to illustrate the effect of common factors. Each functional group represents a predominant ecological role (e.g., offshore surface-feeding birds, demersal fish) within the species group. For the purpose of these guidelines, the most relevant functional groups are coastal top predators, inshore benthic feeders, offshore surface-feeders, inshore surface feeders and offshore (surface or pelagic) feeders.

It is recommended that competent authorities develop a **monitoring strategy**, detailing the species, data, methodology, sites and timeframe. It should also specify the uses of the collected data. Ideally, the monitoring strategy will be implemented through successive multi-annual work plans. It is advisable to keep things simple and aim for the long term; a few species monitored in a reasonable number of representative sites over many (20+) years is likely to provide more informative results than in the case of more ambitious approaches with a variable effort over shorter periods of time.

The choice of monitoring method will depend on the species and data being sought. Counting birds at colonies (**colony census**) is the single most effective way of obtaining numerical information on species abundance and population trends over time. The number of colonies, and their spatial distribution also provides information on species distribution range. Censuses should be carried out regularly every 5 – 10 years and must be done professionally to keep disturbance to a minimum.

Outside of the breeding colonies, **counting bird numbers** at particular sites where birds aggregate (for roosting, bathing, etc.) can provide a good indication of their abundance, especially if censuses are carried out simultaneously at several sites in a particular area. Birds' presence may be influenced by external factors, so good knowledge of local conditions and a large sample size can help improve accuracy of the estimates. Similarly, **shearwater rafts** at sea near the breeding sites can be used as a proxy for breeding numbers at those sites, but there is large variability in the size of those rafts, so they do not necessarily represent differences in population size at the site. This method can complement other techniques, but it is not recommended on its own to estimate bird abundance.

Migration point counts allow for the assessment of the total abundance of birds passing through narrow points at sea. This method can only be expected to provide reliable estimates at a few strategic points like the Strait of Gibraltar but may be less accurate elsewhere. Detectability can be an issue, but it could be improved using distance sampling methods. Counting birds at migration points does not allow to establish a link with national populations, so its use is limited.

Ship-based surveys in set transects at constant speed are a very effective method to monitor seabird distribution and abundance, particularly when the probability of detection is estimated at the same time using the method of distance sampling. Ideally, the surveying team should have free use of a vessel and control over its course of travel and speed. Seabird distribution can be heavily disrupted by the appearance and activity of the survey vessel; fishing boats are the least suitable for surveying, as they tend to attract a large number of species. When surveying, it is recommended to record the activity of the own as well as other vessels, especially if they are fishing.

Aerial surveys are another effective method to study distribution and non-breeding abundance on a large scale but may not be a preferred method in the Mediterranean context. Plane time can be very expensive, and the distance and speed of the survey may limit the ability to detect or identify difficult species. It is important to record all events (e.g., presence of fishing boats) during the surveys. Distance sampling methods should be used to estimate density.

Citizen science (opportunistic observations) and **fishermen questionnaires** are supplementary methods to obtain additional information on seabird distribution. Effectiveness of these methods is limited; their value increases when boat-based observations are provided by regular collaborators and when the exact location (coordinates) is recorded.

Capture–mark–recapture methods are highly effective in providing robust estimates of demo-graphic variables, but they require adequate planning and long-term commitment (at least 5 years, ideally 10 or more), as well as highly specialised teams. This restricts the use of CMR methods to a relatively small number of sites and species. The team should also collect data *in situ* on the breeding biology of the species under study to allow for the development of population models.

Tracking methods are increasingly popular and may be extremely useful to unveil the movements and behaviour of a small number of individuals. However, those individuals may not be necessarily representative of the whole population, so sufficiently large sample sizes may be required. Tracking provides presence-only data at a medium to very high cost; their effectiveness to monitor bird abundance is limited, but they can help find/identify hotspots of seabird activity.

Automated trail cameras can be used to provide data on breeding success and on the causes of failure (e.g., predation). This method is very effective in obtaining information, and multiple cameras can be deployed at several colonies. There are associated costs in the cameras and in the number of human hours required to analyse the images or videos. The use of **drones** allows for the estimation of the total area occupied by the breeding colony, as well as total number and several estimations of density. Some preparation is needed before the start of the breeding season. Surveys should be stopped at the first evidence of disturbance/stress.

Comprehensive censuses should cover all (most) breeding sites and should be carried out regularly, every 5 to 10 years. More intensive work can only be carried out at a few sites at a time: selected sites should be representative of the range of ecological conditions available in the country or region. Also, care is needed when extrapolating to the whole area of results from a few sites.

Survey effort should be timed to coincide with the **peak of detectability** of each species. The biggest effort must be directed at continuing the **time series** of previous monitoring activities. Most statistical analysis methods can cope with one gap in the series, but few can manage two consecutive gaps (seasons) without data.

Use of the monitoring **data** should be defined in the monitoring strategy. Data collection should be straightforward and clear, and it should remain constant for as long as possible, for consistency in the time series. The types of statistical analyses should be clear from the beginning, and they should be shared with the team doing the field work to increase the quality of the data.

Reporting must follow the UN Environment/MAP Barcelona Convention integrated data and information system and should be based on the structure of the Common Indicator Fact Sheets. For EU Member States, the specific reporting scheme of article 12 of the Birds Directive requires them to provide data on the actual state and trends of bird populations, with the next report due in 2019.

1. Introduction

UN Sustainable Development Goal 14 “Life below water” urges to conserve and sustainably use the oceans, seas and marine resources for sustainable development. To achieve this goal, it is necessary to manage human activities and to promote the conservation and wise use of marine ecosystems. Monitoring and assessment, based on scientific knowledge, become indispensable tools in order to assess the status of any marine system and to put in place adequate measures.

The Ecosystem Approach (CBD 2000) integrates the management of human activities and their institutions with the knowledge of the functioning of ecosystems. It requires to identify and take action on influences that are critical to the health of marine ecosystems, thereby achieving sustainable use of ecosystem goods and services and maintenance of ecosystem integrity (Farmer *et al.* 2012). To inform management planning adequately, it is especially important that assessment methods and management tools can incorporate new knowledge, new monitoring methods (to tackle the problem of covering large areas) and indicators into assessments, but still maintain comparability with previous assessments so that any change in the status can be measured and quantified (Borja *et al.* 2016).

2. Policy framework

In the context of the Mediterranean, the United Nations Environment Programme / Mediterranean Action Plan adopted in 2017 its *Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria*, IMAP (Decision IG.22/7). IMAP describes the strategy, themes, and products that the Contracting Parties of the Barcelona Convention are aiming to deliver over the second cycle of the implementation of the Ecosystem Approach Process (EcAp process), between 2016 and 2021, in order to assess the status of the Mediterranean Sea and coast, as a basis for further and/or strengthened measures.

In relation to seabirds, IMAP proposes to monitor and assess the following common indicators:

Common Indicator 3: Species distributional range (EO1);

Common Indicator 4: Population abundance of selected species (EO1);

Common indicator 5: Population demographic characteristics (EO1, e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates)

IMAP recommends monitoring and assessing those common indicators for a selection of representative sites and species, which can showcase the relationship between environmental pressures and their main impacts on the marine environment. For seabirds, these are summarised in Table 1 below:

FUNCTIONAL GROUP	**SPECIES**	
coastal top predators	*Falco eleonorae*	Eleonora’s Falcon
	Pandion haliaetus	Osprey
intertidal benthic-feeders	*n.a.*	
inshore benthic feeders	*Phalacrocorax aristotelis desmarestii*	(Mediterranean) Shag

offshore surface-feeders	*Larus audouinii*	
inshore surface feeders	*Larus genei* *Thalasseus (= Sterna) bengalensis* *Thalasseus (= Sterna) sandvicensis*	Slender-billed Gull Lesser Crested Tern Sandwich Tern
offshore (surface or pelagic) feeders	*Hydrobates pelagicus* *Calonectris diomedea* *Puffinus yelkouan* *Puffinus mauretanicus*	European Storm-petrel Scopoli's Shearwater Yelkouan Shearwater Balearic Shearwater

It is also recommended that the Contracting Parties include at least the monitoring of those species with at least two monitoring areas, one in a low-pressure area (e.g. marine protected area/ Specially Protected Area of Mediterranean Importance (SPAMI)) and one in a high-pressure area from human activity.

In the context of the European Union, Commission Decision (EU) 2017/848 [1] sets the criteria, methodological standards, specifications and standardised methods for monitoring and assessment of biological diversity. It establishes the need to define the criteria, including the criteria elements and, where appropriate, the threshold values, to be used for each of the qualitative descriptors of Good Environmental Status (GES). Threshold values are intended to contribute to the determination of a set of characteristics for GES and inform their assessment of the extent to which it is being achieved. It further establishes that monitoring and assessment should be based on the best available science. However, additional scientific and technical progress may still be required to support their further development and should be used as the knowledge and understanding become available.

3. Species aggregation – functional groups

The use of functional groups for monitoring and assessment purposes results from the work of the Joint ICES/OSPAR Working Group on Seabirds (JWGBIRD) (ICES 2015). Functional groups aim to combine information on different species in order to illustrate the effect of common factors. The rationale for this classification is that it is expected that natural and anthropogenic factors are likely to act similarly on species that share the same food types and display similar feeding behaviours and are those, subject to the same constraints on food availability. Several regional conventions for the protection of the marine environment have adopted the use of functional groups of species (e.g., OSPAR, HELCOM), and they also feature in the revised Commission Decision on the Marine Strategy Framework Directive (2017/848/EU).

IMAP defines functional groups as ecologically relevant sets of species, in particular (highly) mobile species groups, such as birds, reptiles, marine mammals, fish and cephalopods. Each functional group represents a predominant ecological role (e.g. offshore surface-feeding birds, demersal fish) within the species group. For the Mediterranean region, and for seabirds in particular, the most relevant functional groups are:

coastal top predators – birds of prey and other large predators at the top of the food chain in the coastal environment, so not necessarily true seabirds *stricto senso*. In an unperturbed environment, a typical

[1] Commission Decision (EU) 2017/848 of 17 May 2017 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU

representative would be the White-tailed Eagle (*Haliaetus albicilla*), a predator of seabirds, as well as mammals and fish that historically suffered from prosecution and has now become rare in the region. Two other birds of prey, Osprey (*Pandion haliaetus*) and Eleonora's Falcon (*Falco eleonorae*) typically nest on sea cliffs. Although ecologically their niche may be broader, they are considered to belong to this group for monitoring and assessment purposes.

intertidal benthic-feeders – typically shorebirds (including Spoonbill *Platalea leucorodia*), ducks, geese, swans and gulls that mostly walk or wade while feeding. In the Mediterranean region, such birds generally associate with wetlands or saltpans, rather than being characteristically coastal or marine. IMAP does not identify any particular species as belonging to this functional group, so none will not be considered for these Guidelines.

inshore benthic feeders – birds that dive to the seabed to feed, generally on demersal fish. In the Mediterranean region, this group is best represented by the Mediterranean Shag (*Gulosus (=Phalacrocorax) aristotelis desmarestii*), an endemic form estimated to number only 10,000 individuals and showing a comparatively local distribution. Mediterranean Shags have historically suffered a succession of declines and recoveries and may be heavily affected by human pressure, both as a result of habitat occupation and of bycatch in fisheries.

offshore surface-feeders – birds (e.g., gulls) that feed in the top layer of the water column on the outer part of the continental shelf or in the open sea. The Mediterranean endemic Audouin's Gull (*Larus audouinii*) is the most characteristic species of this functional group in this region. The species was once rare but has seen a substantial recovery (especially in the western Mediterranean), as a consequence of the increased availability of fishing discards and of the protection of its nesting habitat.

inshore surface feeders – restricted as feeders to the surface layer of the water column and occurring mostly near the shore. In the Mediterranean region, this niche is occupied by the Slender-billed Gull (*Larus genei*), Lesser Crested Tern (*Thalasseus (= Sterna) bengalensis*) and Sandwich Tern (*Thalasseus (= Sterna) sandvicensis*). The former two, whilst not being endemic as species, have geographically and numerically significant populations in the Mediterranean. Their specialised association to low-lying coasts and shallow waters has traditionally made them vulnerable to habitat transformation.

offshore (surface or pelagic) feeders – open seas are typically the realm of seabirds that feed across a broad depth range in the water column (albatrosses, petrels, penguins). In the Mediterranean, they form a small group of endemic species that are extremely important for conservation: the Balearic Shearwater (*Puffinus mauretanicus*) and the Yelkouan Shearwater (*Puffinus yelkouan*) are both globally threatened. Together with Scopoli's Shearwater (*Calonectris diomedea*), which is also endemic, they fall frequent victims to bycatch in longline fisheries and are also threatened on land by introduced predators in their breeding colonies. The European Storm-Petrel (*Hydrobates pelagicus*) is the sole representative in our region of the cosmopolitan group of storm-petrels; these are small but long-lived and truly oceanic seabirds that feed on plankton and act as effective indicators of the general state of the marine environment.

4. Monitoring strategy

For effective use of limited resources, it is crucial that competent authorities develop a monitoring strategy, which can provide detail on important aspects such as species, sites, methods and timing and regularity. It is also important to decide on the uses of the collected data. Ideally, the strategy will be implemented through successive multiannual work plans that will integrate pre- and post-field work, as well as the development of the monitoring activities that need to be undertaken.

Based on the species composition, area and available resources, a monitoring strategy should cover the following aspects:

a) **Species** – as a minimum, the representative species of each functional group (Table 1) should be monitored on a regular basis, if present in the country. It is possible to add more species to the mix, but such a decision must take into account that effective monitoring requires a long-term commitment, which may be difficult to meet for prolonged periods of time. Also, the decision to monitor additional species should not put at risk the monitoring of the standard species set, as these benefit from the fact that they are being monitored on a wider scale (e.g., whole Mediterranean region), which adds value to the data obtained at national or local scale.

b) **Data** – the nature of the data to be collected varies with the common indicator and is specified in the Common Indicator factsheets. A monitoring strategy should consider possible data in the form of numerical values of distribution (total area occupied, number of squares, maps), abundance (number of birds present, number of apparently occupied nests, etc.; relative density), breeding productivity (young fledged per egg laid, young fledged per breeding attempt) and general demography (annual survival rate, juvenile recruitment rate, age class ratio).

c) **Methodology** – an assessment of population size can be obtained either by counting the total number of individuals at a given time or by counting numbers at selected periods of sampling, and then calculating the total number through extrapolation. The latter method (i.e., sampling + calculating) is by far the commonest, but it requires an appropriate design of the sampling periods / sites, plus the use of robust statistical methods for the calculation. A monitoring strategy should be specific about the sampling methods, the monitoring techniques and the calculation procedures. It should also describe how different methods should interact, e.g. by calculating an annual population trend value (through stratified and representative sampling) and combining with a comprehensive, large-scale census every 5 or 10 years.

d) **Sites** – the monitoring strategy shall define the spatial dimension of its sampling effort. Whole-area censuses can only be carried every number of years (usually, between 5 and 10), whereas the annual effort of obtaining data on population trends or on breeding performance will have to be limited to a smaller sample of representative sites. Even within single (large) colonies, it is often necessary to obtain detailed data from a randomised selection of squares. The number and location of colonies monitored will influence the results [2], so it is important that the strategy considers the representativeness of each site in relation to the general context. It is generally recommended to treat the data with robust statistical methods that bear in mind the relative weight of each site in the wider context of the entire population.

e) **Timeframe** – the timing and repeatability of monitoring activities will vary according to species and area. In general, the monitoring strategy should aim at obtaining data *ad infinitum*, or at least for as long as threatened species or sites remain in that status. For that reason, the strategy should aim at obtaining the most valuable data (e.g., overall productivity with preference over first egg laying date), and the multiannual work plan should guarantee that the necessary monitoring takes place at least once every year. For effective monitoring, the strategy should also take into account the issue of seasonality and propose

[2] Tobler's first law of Geography (spatial autocorrelation) applies: "Everything is related to everything else, but near things are more related than distant things" (Tobler 1970).

the ideal timing for each sampling to take place. Ideally, the work plan should seek to optimise and combine samplings for different species, wherever possible, to maximise the outcome.

In general, it is advisable to keep things simple and aim for the long term; a few species monitored in a reasonable number of representative sites over 20+ years is likely to provide results that are far more informative than in the case of more ambitious approaches with a variable effort over shorter periods of time.

5. Monitoring methods

The choice of monitoring method will depend on the species and data being sought. For seabirds in the Mediterranean region, the following methods may be considered:

Colony census

- All seabirds invariably need to visit land in order to nest, and most breed colonially. Counting birds are colonies is the single most effective way of obtaining numerical information on their abundance (Common Indicator 4), and thus of their population trends over time. The number of colonies, and their spatial distribution also provides information on species distribution range (Common Indicator 3).

- In medium (250-1000 breeding pairs) to large colonies (> 1000 b.p.), it will be difficult to accurately assess the exact number of birds present. In these cases, it is recommended to record and plot the entire area of the colony (e.g., by using drones, see below), and to monitor the spatial evolution of the colony over time.

- For very large colonies (e.g., > 5000 b.p.), it is recommended to define smaller squares (e.g., 20 x 20 m, 50 x 50 m, 100 x 100 m or larger, depending on the species and the geography of the site) and to count every single nest inside the square, to obtain a measure of density. By repeating the same procedure on a number of squares, it is possible to obtain a measure of the average density, as well as its standard deviation. Such values can be used to calculate the total population of the colony, by multiplying the total number of squares by the average density ± standard deviation.

- For burrow-nesting species (storm-petrels, shearwaters), it is good practice to estimate the average number of nests per burrow, as a single burrow or cave may contain several breeding pairs or nests.

Land-based roost (aggregation) counts

- Several species, particularly of gulls, terns and cormorants (shags), aggregate at predictable sites after feeding or for roosting, bathing, etc. Assessing bird numbers at those sites can provide a good indication of their abundance (Common Indicator 4), especially if censuses are carried out simultaneously at all sites where birds aggregate in a particular area. This method is not without its drawbacks, as bird presence may be influenced by external factors such as weather, season, day of the week, etc., so good knowledge of local conditions and a large sample size can help improve accuracy of the estimates.

- Similarly, the well-known tendency of some seabirds, particularly shearwaters, to form rafts at sea near the breeding sites can be used as a proxy for breeding numbers at those sites. It is also known, however, that there is large variability in the size of those rafts, due to weather, time of year and local characteristics of each colony, so they do not necessarily represent differences in population size at the site.

Migration point counts

- As birds travel between different areas (e.g., during migration), geography may force them to funnel through certain narrow points, where they become easier to detect and to count. One such place in the Mediterranean region is the Strait of Gibraltar, the only connection between the Mediterranean Sea and the Atlantic Ocean and a necessary gateway for all species whose populations move between the two. A small number of similar places exist in the region (e.g., Bosphorus, Dardanelles, northern Tunisia, strait of Otranto) but their accuracy in tracking bird numbers is probably less reliable. Bird abundance passing on migration near such places can be used as a proxy for their total abundance (Common Indicator 4). However, issues of detectability (only a proportion of all birds passing near the watchpoints can be seen from land) and representativeness (the breeding sites of passing birds cannot be known) make this method not entirely suitable for monitoring seabirds in the Mediterranean. Combined analyses of all watchpoints on a regular (annual) basis, and a long time series, may be able to reflect real population changes.

Ship-based surveys

- Systematic surveying of marine areas in search of seabirds has historically produced good results in the detection of hotspots of activity, generally associated to foraging behaviour. Observations of seabirds in set transects at constant speed are particularly useful if the probability of detection is estimated at the same time using the method of distance sampling (Buckland et al. 2001). This method allows for the estimation of the density of each species per transect (or per fraction of transect). Multiple estimations of density can be combined and averaged for each unit of space (e.g., 10 x 10 km or 1° x 1° cells), so they can be mapped and analysed spatially. This provides useful values of bird distribution (Common Indicator 3) and abundance (Common Indicator 4).

- This well-known method requires free use of a vessel that can offer good visibility, ideally with vantage points as used for cetacean surveys; line ferries are used in several places with positive results, but their inability to change course limits their effectiveness for seabird monitoring. Seabird distribution can be heavily altered by the appearance and activity of the survey vessel; fishing boats are the least suitable for this purpose, as they tend to attract a large number of species. When surveying, it is recommended to record the activity of the own as well as other vessels, especially if they are fishing.

Aerial surveys

- Similar to ship-based surveys but on another scale, aerial surveys are used to collect distribution and abundance data on seabirds, particularly of species with high detectability (e.g., gannets Morus sp.) or low mobility (e.g., auks Alcidae). Using distance sampling methods, aerial surveys can provide abundance data over large sections of the ocean and are thus quite effective, albeit expensive. However, in the Mediterranean region and for our set of species, aerial surveying is arguably not the most suitable method. Detectability can be potentially quite low (e.g., of storm-petrels, shearwaters) and identification at species level may be very difficult, almost impossible in some cases (e.g. Balearic vs. Yelkouan Shearwater, or Sandwich vs. Lesser Crested Tern).

Citizen science (bird portals, logbooks, opportunistic observations)

- Opportunistic observations of seabirds collected non-systematically by amateur ornithologists, seafarers or the general public can provide additional information on bird distribution (Common Indicator 3). Such data can rarely be used to estimate densities, and therefore abundance, because they generally lack essential information on the space covered (transect) or the observation effort (time). Their value lies in their ability to provide information on spatial distribution and is particularly useful in detecting change in the distribution of rapidly expanding species.

Questionnaires (fishermen, seafarers)

- Through the use of questionnaires, it is possible to obtain useful information from fishermen or professional seafarers. The value of this information is generally qualitative and not quantitative, so it is most useful when it involves data on seabird distribution (Common Indicator 3), particularly on the location of nesting sites / colonies. Occasionally, the collaboration of fishermen can provide additional info on breeding phenology or success, and thus contribute to Common Indicator 5 (demography).

Capture – Mark – Recapture

- Capture – mark – recapture (CMR) methods provide robust estimates of demographic variables such as individual survival, recruitment and emigration (Amstrup, McDonald & Manly 2005). They require adequate planning and long-term commitment, because seabirds are generally long-lived. For this activity, highly specialised teams are required that can capture and ring a sufficiently large number of birds over a long sequence of years (at least 5 years, ideally 10 or more), and who can analyse the data using specific software (Program MARK: White & Burnham 1999). This restricts the use of CMR methods to a relatively small number of sites and species.

- In most cases, the same team of professional biologists collect data in situ on the breeding biology of the species under study (e.g., no. of eggs laid, hatching success, chick survival, breeding success) that add to the information on demography and are essential for the development of population models.

Use of tracking methods (VHF, GPS, PTT) to locate important sites

- With the development of tracking technologies, the movements and behaviour of many individuals of several seabird species have been unveiled. In the Mediterranean region, the most intensively studied species with this method are Scopoli's and Yelkouan Shearwaters, Audouin's Gull, Eleonora's Falcon and Osprey. Tracking only provides information about the unique movements of tagged individuals, so a large sample size may be needed to extrapolate those movements to the rest of the population. Despite the limitations, tracking data can be particularly useful in assessing the distribution of birds in a population or in finding their breeding sites (e.g., the discovery of new colonies) (Common Indicator 3). On the negative side, this method is expensive and can only provide presence-only data from a fraction of the population.

Trail cameras

- Automated trail cameras can be situated strategically at nesting sites to obtain timed data about breeding biology and behaviour with limited disturbance. Importantly, trail cameras can also provide data on breeding success and on the causes of failure (e.g., predation). This method is very effective in obtaining information, and multiple cameras can be deployed at several colonies. However, there are associated costs in the cameras themselves and in the number of human hours required to go through the recorded images or videos.

Drones

- The use of drones to assess breeding numbers at a given site is increasingly popular and constantly being developed. This method allows for the estimation of the total area occupied by the breeding colony (Common Indicator 4), as well as total number and several estimations of density if the necessary arrangements have been put in place before the birds settle to start breeding (see Sardà-Palomera et al. 2017). For asynchronous species (e.g., Eleonora's Falcon) it may useful to survey the colony several times in order to obtain data from all phases of the breeding cycle and count in all nesting attempts.

6. Territorial coverage

A monitoring strategy should recommend the spatial scale of the monitoring effort – should all areas be monitored all the time? Or, given limited resources, is it better to concentrate on a few sites and extrapolate to the whole? The answers to these questions depend on the geographical characteristics, and on the species being monitored. In general, it is advisable to carry out regular censuses that cover all (most) breeding sites and attempt to count all the birds; such censuses should be carried out regularly, every 5 to 10 years.

For more intensive work, such as a capture–mark–recapture scheme, or monitoring with trail cameras or drones, work can only be carried out at only a few sites at a time. In the selection of those sites, it is important to follow two criteria: (i) the sites should be representative of the range of ecological conditions available in the country or region, so that good sites as well as not-so-good sites are included; and (ii) extrapolation to the whole area of results from a few sites must be done with care because that the country is likely to be ecologically diverse.

7. Sampling design and representativeness

To obtain precise estimates, it is necessary to plan the sampling effort adequately. This is particularly important when the whole area cannot be surveyed and only a selection of squares (cells) can be visited to obtain data. Survey effort should cover a sufficient number of cells that (a) represents the entire spectrum of ecological conditions, and (b) is statistically robust to allow for analysis of the data. The same strategy applies to the local scale, in choosing the number of squares to count nests in a large breeding colony, or on a large scale, in surveying marine areas using transects.

Sampling should take place over enough cells, and preferably in the same cells or transects, every time. Through this spatial consistency, a data log of bird counts at each spatial unit will develop over time that will allow for further analysis in the future, if conditions change.

8. Timing and regularity – the importance of long-time series

Survey effort should be timed to coincide with the peak of detectability of each species, for optimal results. Peaks of breeding activity vary seasonally and often during the course of the day for all species, and a monitoring strategy should account for that variability whilst trying to integrate different monitoring activities into a single work plan. In any case, it is important to record all relevant details (day of week, time of day, activity of fishing vessels, disturbance events, etc.) when carrying out the surveys, so that they can be taken into account during the analysis of the data.

The value of monitoring becomes increasingly important as the time series becomes longer, because the ability to detect change also increases. Therefore, the biggest effort must be directed at continuing the time series of previous monitoring activities, which must remain unaltered with the same methods and in the same places unless there is good reason to change.

Most statistical analysis methods can cope with one gap in the series (generally equivalent to one season without monitoring), but few can manage two consecutive gaps (seasons) without data. Time series interrupted in this way are generally irreparable and end at that point.

9. Data management, analysis and control

Use of the monitoring data should be defined in the monitoring strategy. This aspect should be integrated in the design of all monitoring activities, and it should be taken into account when they are carried out. Data collection should be straightforward and clear, and it should remain constant for as long as possible, for consistency in the time series. Ideally, a data analyst should form an integral part of the monitoring team, and they should be able to inform survey design. This strategy will improve the overall efficiency of the team.

The types of statistical analyses should be clear from the beginning, and they should be shared with the team doing the field work. With an increased understanding of the whole process, individual observers will put more attention into collecting additional or supplementary data about the conditions at the time of conducting their activity; this will increase the quality of the data.

10. Reporting

As part of IMAP's integrated assessment, Contracting Parties to the Barcelona Convention are required to report on the quality and status of the marine environment under their jurisdiction. Reporting must follow the UN Environment/MAP Barcelona Convention integrated data and information system and should be based on the structure of the Common Indicator Fact Sheets. IMAP encourages Contracting Parties to use up-to-date tools for data exchange.

In the context of the European Union, article 12 of the Birds Directive 2009/147/EC (EU 2009) requires that EU Member States report on the implementation of the national provisions taken under this Directive. This includes providing data on the actual state and trends of bird populations, and must be done every six years, starting in 2013, so the next report is due in 2019. The Birds Directive applies to all species of naturally occurring birds in the wild state in the European territory of the Member States, and a detailed report has to be completed for all regularly occurring species in the relevant seasons, including breeding, wintering and passage.

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Annex I Comparative table.: Characteristics of monitoring techniques

Monitoring technique	Suitable species	Common Indicator(s)	Personnel requirements	Equipment	Recommendation
Colony census	all	4 – abundance (3 – distribution range)	trained staff/volunteers; at least one team (2-3 people) per colony; ideally several teams working simultaneously in several colonies; coordination	boat to access islands or difficult places; binoculars; camera / drone	▪ single most effective technique; ▪ should be carried out regularly every 5 – 10 yrs; ▪ must be done professionally to keep disturbance to minimum
Land-based roost (aggregation) counts	*Puffinus* (rafts) *Calonectris* (rafts) *Phalacrocorax* *Larus* *Sterna*	4 – abundance	single trained observer or, preferably one team (2-3 people) per site; ideally, several teams working simultaneously in several sites; coordination	binoculars / telescope; access to viewing points	▪ no substitute for colony census (especially true for shearwater rafts) ▪ suitable for non-breeding species ▪ weather, season and local conditions may affect numbers ▪ should be repeated regularly
Migration point counts	*Puffinus* *Calonectris* *Larus* *Sterna*	4 – abundance	trained observers; at least one team (2-3 people) per watchpoint; ideally several teams placed strategically to maximise cover	binoculars / telescope; access to viewing points	▪ reliable estimates only expected at few places like Strait of Gibraltar, Bosphorus, etc. ▪ no link to breeding (national) populations

					▪ partial detectability; could be improved by using distance sampling
Ship-based surveys	all	3 – distribution range 4 – abundance if additional data taken	1-3 trained observers to cover 180° view; binoculars	vessel with good visibility (e.g. for watching cetaceans); control over vessel course/speed of travel; binoculars	▪ very effective method to study distribution and non-breeding abundance ▪ vessel time very expensive, so less optimal solutions often used ▪ ability to fix course/speed of travel needed for density estimation ▪ fishing boats change bird distribution and behaviour and should be avoided ▪ important to record all events (e.g., presence of fishing boats) during survey
Aerial surveys	most species	3 – distribution range 4 – abundance	1-2 trained observers to cover 180° view; binoculars	low-speed aeroplane with good visibility; control over plane course/speed of travel; binoculars	▪ effective method to study distribution and non-breeding abundance on large scale ▪ plane time very expensive ▪ ability to fix course/speed of travel needed for density estimation ▪ distance/speed limits ability to identify difficult species

					▪ important to record all events (e.g., presence of fishing boats) during survey
Citizen science (bird portals, logbooks, opportunistic observations)	all	3 – distribution range	volunteers with varying degrees of training		▪ low effectiveness; only supplementary info expected ▪ most valuable data from boat-based observations ▪ important to record exact location (coordinates)
Questionnaires (fishermen, seafarers)	all	3 – distribution range (5 – demography)	volunteering professionals; interviewing staff		▪ limited effectiveness ▪ value increased when collaboration becomes well established over time
Capture – Mark – Recapture	all	5 – demography (4 – abundance)	professional team (2-3 people) with ringing licence; data analyst	ringing equipment; access to colonies	▪ very effective method to obtain demographic data ▪ monitoring must be maintained for >5 yrs ▪ work at breeding colonies should can be combined with collection of data on breeding biology for comprehensive demographic analyses
Tracking methods (VHF, GPS, PTT) to locate important sites	all	3 – distribution	professional team (2-3 people) with ringing licence; data analyst	tagging devices; ringing equipment; access to colonies	▪ extremely useful method to unveil individual movements / behaviour ▪ not necessarily representative of whole

					population, so large sample size required ▪ presence-only data ▪ medium to very high cost
Trail cameras	all	5 – demography	small professional team (1-2 people); image/video analyst	trail cameras (several); access to site	▪ can be used to provide data on breeding success and causes of failure (e.g., predation) ▪ effective and relatively low cost, but require long man hours of lab work analysing images/footage ▪ useful as supplementary method ▪ low disturbance
Drones	all	3 – distribution 4 – abundance if additional data taken	small team (1-3 people) with licence to fly drone; image/video analyst	flying drone; HD camera	▪ very useful to assess total area of breeding colony (for estimation of density) ▪ some preparation before breeding season essential ▪ survey should be stopped at first evidence of disturbance/stress

D. Guidelines for monitoring marine turtles in the Mediterranean

Contents

ACRONYMS AND ABBREVIATIONS

ASM	Age at Sexual Maturity
CCL	Curved Carapace Length
CF	Clutch Frequency
CI	Confidence Intervals
CMR	Capture-Mark-Recapture
CS	Clutch Size
DE	Number of dead embryos
EES	Number of empty egg shells
ES	Emergence Success
GI tract	Gastro Intestinal Tract
GPS	Global Positioning System
IP	Incubation Period
IUCN	International Union of Conservation of Nature
PE	Number of predated eggs
PIT	Passive Integrated Transponders
RMI	Remigration intervals
RMU	Regional Management Units
RNI	Re-nesting (inter-nesting) intervals
SCL	Straight Carapace Length
SSF	Small-Scale Fleets
TED	Turtle Excluder Device
UAV	Unmanned Aerial Vehicle
UE	Number of unfertilized eggs

1. INTRODUCTION

Two species of sea turtle – the loggerhead turtle and the green turtle – regularly occur and breed in the Mediterranean Sea. The breeding activities of both species are regularly monitored in the main nesting areas of ten countries; namely, Cyprus, Egypt, Greece, Israel, Italy, Lebanon, the Libyan Arab Jamahiriya, the Syrian Arab Republic, Turkey and Tunisia. The species' distributional range, population abundance and demographic characteristics are generally estimated according to nest counts in those above countries. A recent approach has been to divide all species of sea turtle into Regional Management Units (RMU; Wallace et al. 2010), identifying Mediterranean RMUs for loggerhead turtles (RMU:11) and green turtles (RMU:17).

Sea turtles are a long-lived species; they can take more than two decades to reach maturity. They also use different habitats at different age classes. Post-hatchlings mainly use pelagic habitats as developmental areas and remain offshore until they reach large juvenile size (<40cm Curved Carapace Length (CCL). However, once their CCL exceeds 30 cm, they start to shift their developmental areas to neritic habitats. The monitoring of sea turtles must therefore be conducted not only on beaches but also in the water, as they migrate between feeding grounds and spend the winter months.

The monitoring of sea turtles is mostly performed using these techniques: **(i) counting the number of nests during nesting period, (ii) collecting stranded turtles, (iii) in-water capture-mark-recapture studies, and (iv) boat and aerial surveys.**

Nesting female sea turtles and their clutches in particular, have been used as indicators of population size and trends (Bjorndal et al., 1999; Broderick et al., 2002; Margaritoulis, 2005; Türkozan & Yilmaz, 2008). Nesting activity has the potential to address two indications that specifically relate to the Barcelona Convention Decision on Common Indicators (IG.22/3), namely:

- Common indicator 4 (CI4): Population abundance of selected species
- Common indicator 5 (CI5): Population demographic characteristics

Sea turtles inhabit the shallow waters along coasts and around islands, but most are highly migratory, particularly as juveniles, and are found in the open sea. After the nesting season, species in temperate areas migrate to warmer waters, to avoid cold temperatures. In addition, only female turtles are observed on the nesting beaches; males and juveniles never come ashore (Heppell et al., 2003). Consequently, determining empirical estimates for the number of juveniles is extremely challenging.

For instance, boat surveys and aerial surveys can be used to estimate the number of turtles on the surface as Visual Counting Surveys and then the total number can be extrapolated. These techniques give an indication in accordance with the Barcelona Convention Decision (IG.22/3), in particular:

- Common indicator 3 (CI3): Species distributional range

These monitoring activities can be classified as: 1- Monitoring carried out on beaches; 2- Monitoring carried out at sea and 3- Monitoring that takes place in rehabilitation centres and/or labs.

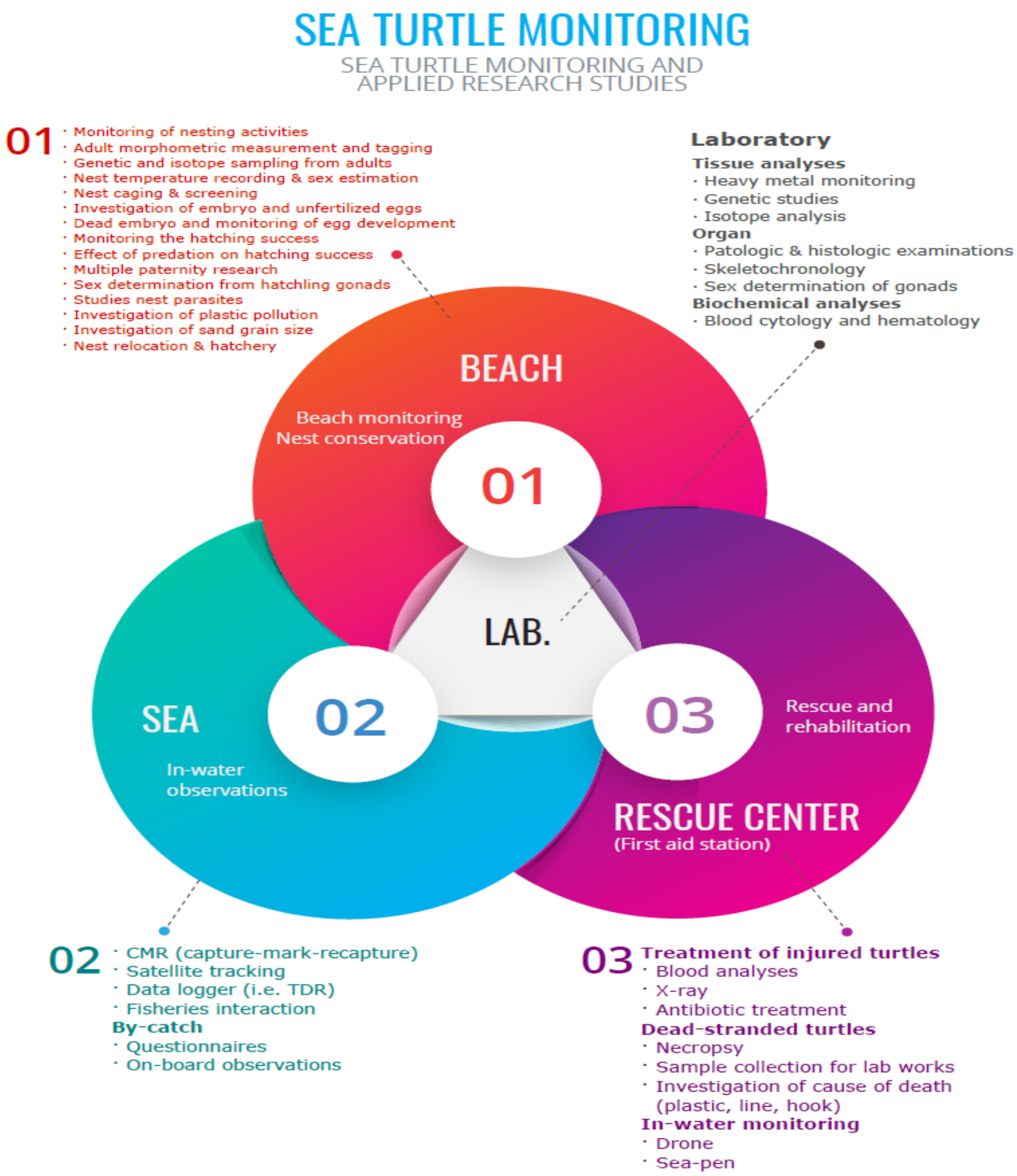

Figure 1. Spatial sea turtle monitoring and research activities

Sea turtles exhibit high nest-site fidelity. Research on migratory behaviour and the distribution of sea turtles shows that adult turtle fidelity to breeding sites is also a component of homing behaviour. It has also been directly observed, mainly in females, through flipper and satellite tagging (Margaritoulis, 1998; Broderick et al., 2003; Casale et al., 2013; Schofield et al., 2013). Site fidelity is even stronger in adults, as they appear to return to the same foraging ground after reproductive migration (Godley et al., 2003; Lazar et al., 2004; Broderick et al., 2007; Zbinden et al., 2008; Schofield et al., 2010a; Schofield et al., 2010b; Casale et al., 2013). Site fidelity can be monitored using standard flipper tagging and satellite tagging.

1.1. Distribution Ranges of Sea Turtles

1.1.1. Nesting Site Distribution of Loggerhead Turtles

Loggerhead turtle (*Caretta caretta*) nesting occurs over a wide area, with more than 96% of clutches laid in Cyprus, Greece, Libya and Turkey, which host the major nesting rookeries for this species in the Mediterranean. Lower levels of nesting take place on the Mediterranean shores of Egypt, Israel, Italy, Lebanon, Syria and Tunisia, with minor and infrequent nesting occurring along the western basin coastline of France, Italy, Spain and their offshore islands. Sporadic nesting is also recorded on the Aegean coast of Turkey and on the coast of Albania. If all the surveyed years are included, there is an average total of 6751 loggerhead turtle clutches per year, with 8179 in more recent times (Casale et al., 2018).

1.1.2. Nesting Site Distribution of Green Turtles

Green turtle (*Chelonia mydas*) nesting is restricted to the eastern Mediterranean and has only been recorded in Crete, Cyprus, Egypt, Israel, Lebanon, Syria and Turkey. There are 13 major nesting locations with an average of 1650 green turtle clutches per year, if all surveyed years are included and 2204 in more recent times (Casale et al., 2018). The principal green turtle rookeries are located in Cyprus, Syria and Turkey with minor nesting aggregations occurring in Egypt, Israel and Lebanon. The nesting sites in Turkey and Cyprus account for more than 90% of all green turtle nesting in the Mediterranean.

1.2. Population Abundance and Trends

The first parameter that needs to be analysed is population abundance and its trend in nesting populations. The nest counts and number of females nesting on the beaches, as mentioned above, need to be recorded using the same methodology. The population abundance in the sea has to be determined via in-water observations.

Loggerhead turtle: A more accurate comparison between past and current nest counts at 16 index nesting sites, which was included in a recent IUCN Red List assessment of the Mediterranean loggerhead turtle subpopulation as an RMU, reported a positive trend and was classified as of Least Concern (Casale, 2015). The abundance of adult females on the beach can be calculated from nest counts, clutch frequency (the number of clutches laid by a female in a nesting season), remigration intervals (the number of years between two consecutive nesting seasons) and adult sex ratio. The most recent available data provides an average of 8179 nests per year at the monitored nesting sites (Casale et al., 2018) and estimated 15843 adults (CI95%: 6915-31,958) (Casale and Heppell, 2016). Abundance estimates at sea, where juveniles represent the majority of the population, have been conducted through several spatially limited aerial surveys. Casale and Heppell (2016) attempted to provide at least the order of magnitude of a possible range of values for the total population abundance (including adults): from 1,197,087 (CI95%: 805,658-1,732,675) to 2,364,843 (CI95%: 1,611,085-3,376,104).

Green Turtle: For green turtles, a rough comparison of average nest counts at seven nesting sites between the same two arbitrary periods described above, indicates an overall positive trend. In Cyprus, an increasing proportion of neophytes (nesting females captured for the first time and assumed to be in their first year of breeding) was observed (Stokes et al., 2014), suggesting an increasing population. Monitoring programmes for green turtles at sea have yet to be established.

The most recent available data provides an average of 2204 nests per year at monitored nesting sites (Casale et al., 2018). Casale and Heppell (2016) estimated 3390 adults (CI95%: 1894-6552) with a population abundance from 261,727 (CI95%: 176,284-391,386) to 1,252,283 (CI95%: 679,433-2,209,833).

1.3. Population Demographics

Population demographic parameters need to be collected from nests and nest environments, as well as from in-water observations.

1.3.1. Monitoring of Development and Incubation Period

The monitoring of nests and embryos are also important and vary among the beaches. The incubation duration of clutches negatively correlates with nest temperature for both species of sea turtle (Godley et al., 2001a; Mrosovsky et al., 2002; Kaska et al., 2006) and is highly variable among the Mediterranean beaches. For example, viable hatchlings from loggerhead nest temperatures as low as 26.5 ºC (with an incubation duration up to 79 days) have been recorded in Sicily, Italy (Casale et al., 2012a), whilst the longest incubation duration for loggerhead turtles in the Mediterranean (89 days) has been recorded twice on Marathonissi beach (Laganas Bay, Zakynthos) (Margaritoulis, 2005; Margaritoulis et al., 2011). At the opposite end of the temperature range, nest temperatures as high as 33.2ºC in Cyprus (Godley et al., 2001a) and with an incubation duration as short as 36 days in Calabria, Italy (Mingozzi et al., 2007) have been observed. Nest temperature measurements have also been carried out for green turtles and the nests were usually deeper than those of loggerhead turtles (i.e., Kaska et al., 1998; Candan & Kolankaya, 2016).
The parameters that need to be monitored here are as follows:

- Inter-nesting (or re-nesting) intervals (RNI) which is between 12.7-19.9 days,
- Remigration intervals (RMI),
- Clutch frequency (CF), the number of clutches deposited by a female in a single season,
- Incubation periods (IP),
- Hatchling sex ratios and,
- Hatching success and hatchling emergence success (ES%).

1.3.2. Recording the Clutch Size and Hatching Success

For loggerhead turtles in the Mediterranean, substantial differences exist in terms of clutch size, with the smallest females and clutch sizes observed in Cyprus and the largest females and clutch sizes observed in Greece. The number of clutches laid per season range between 1–5 clutches per season for loggerheads at Alagadi, Cyprus (Broderick et al., 2003) and this parameter could be associated with re-nesting interval. The mean clutch size for loggerhead turtles ranges from 64.3 to 126.8 eggs, among the different Mediterranean sites.
The mean clutch size among the different Mediterranean sites ranges from 108 to 120 eggs for green turtles (see references in Casale et al., 2018).
The monitoring and recording of nest depth, diameter, humidity, hatching success, clutch size, fertilization rates and mortality rates is essential.

1.3.3. Spatial and Temporal Monitoring of Sex Ratio

The sex ratio of hatchlings on the beaches and the sex ratios in adult and sub adult stages are important when monitoring the population of both sea turtle species. When estimating the sex ratio of the hatchlings, the most commonly used methods are nest temperature measurements and gonad histology. Laparoscopy can also be used for hatchlings and at later ages. The monitoring of the temporal and spatial changes of the sex ratio on the beaches is also very important when taking the possible effects of global warming into account.

1.3.3.1. Loggerhead turtle sex ratio estimations

The pivotal temperature (the egg incubation temperature at which both sexes are produced in equal numbers) for Mediterranean loggerheads assessed in laboratory and field conditions, is about 29-29.3°C and is similar to other populations elsewhere, with a pivotal incubation duration (at which both sexes are produced at equal numbers) of 53 days from laying to hatching (Kaska et al., 1998; Mrosovsky et al., 2002). Other studies carried out under natural conditions, (Fuller et al., 2013) found a slightly lower (28.9ºC) pivotal temperature and a longer incubation duration than expected (56.3 days), due to the effect of metabolic heating generated by the whole nest.

By applying different indirect sex determination methods, loggerhead hatchling production at most Mediterranean nesting sites are likely to be highly female-biased, with the major rookeries in Greece, Turkey, Libya and Cyprus producing 60-99% females (see references in Casale et al., 2018). Interestingly, gonadal histology as a direct sexing method, although possibly biased by the field sampling protocols and applied only in a limited number of cases, showed less skewed loggerhead hatchling sex ratios (55.6-79% females). Conversely, male-biased hatchling production occurs in some sites, such as Marathonissi beach in Zakynthos, Greece (Margaritoulis, 2005; Zbinden et al., 2007; Margaritoulis et al., 2011) and Kuriat Island in Tunisia (Jribi & Bradai, 2014) and in some years may also be possible at other sites.

Spatio-temporal variations in sex ratios have also been reported (Kaska et al., 2006; Katselidis et al., 2012; Fuller et al., 2013), with more male hatchlings being produced from the nests laid at the beginning and the end of nesting season (May and August, respectively), than from those laid in the middle of nesting season (June-July). Eggs at the top of a nest are also likely to be exposed to more heat from the sun and produce relatively more females than those at the bottom of a nest (Kaska et al., 1998). Beach sand colour (albedo), sand grain size and shading by vegetation are all important factors when determining hatchling sex ratios (e.g. Kaska et al., 1998; Hays et al., 2001; Zbinden et al., 2007; Fuller et al., 2013).

1.3.3.2. Green Turtle sex ratio estimations

Clutch temperatures in green turtle nests range from 28.3 ºC with an incubation period of 59 days in Turkey (Candan & Kolankaya, 2016) and as high as 32.5 ºC and an incubation period of 43 days in Cyprus (Kaska et al., 1998; Broderick et al., 2000). Mean incubation durations range from 49 to 60 days (Casale et al., 2018). Primary sex ratios tend to be female-biased (70-96% females; (see references in Casale et al., 2018). An operational sex ratio of 1.4M:1F was estimated from a paternity study at Alagadi (Alagati) Beach, Cyprus (Wright et al., 2012).

1.3.3.3. In-Water Sex Ratio Estimations

Surprisingly, and contrary to predominant female-biased hatchling production, the sex ratios of juvenile loggerhead turtles in most Mediterranean marine habitats showed no significant deviation from a 1:1 ratio, with the proportion of females ranging between 52 and 56%. The explanation initially given for the discrepancy between strong-female biased hatchling production and almost even sex ratios in juvenile loggerheads was the strong male-biased immigration of Atlantic juveniles into the Mediterranean Sea (Casale et al., 2002; Casale et al., 2006). Overall, a female bias in the juvenile sex ratio (1.56:1) was recorded in the long-term study in the Tyrrhenian Sea, although in some years this ratio has shown no deviation from a 1:1 ratio (Maffucci et al., 2013).

1.3.3.4. Monitoring the Effects of Global Warming

Temperature profiles of monitored nesting beaches in the eastern Mediterranean strongly imply a female biased sex ratio for hatchlings (Casale et al., 2000; Godley et al., 2001a; Godley et al., 2001b; Kaska et al., 2006; Zbinden et al., 2007; Fuller et al., 2013). In the context of global warming, even more female-biased hatchling sex ratios may result. However, extremely skewed sex ratios resulting from a moderate increase of incubation temperature may not necessarily be negative for the population dynamics and a greater threat is represented by reduced hatching success at higher temperatures (Pike, 2014; Hays et al., 2017).

Measuring nest and sand temperature offers simple and reliable data for sex ratio estimation, a technique for which electronic data loggers are commonly used. Measuring the sand temperature provides information about the general profile of a beach but *metabolic heating* (the heat that embryos produce during incubation) should also be taken into account, as this usually means the nest temperature is higher than that of the surrounding sand.

In order not to interfere with the nest after nesting, the best time for placing data loggers is during egg laying. The data logger may be placed at the bottom or the top of the nest, but the most common practice is to place it in the middle of the nest.

If a nest is found after the eggs have been laid, the data logger can only be placed in the nest within the first 24 hours of egg laying. Follow the same procedure during nest relocation, when removing the eggs from the nest and returning them. Data loggers can be collected during the nest excavation. Data loggers, their launching, placement into the nest, information retrieval and the downloading of temperature data can be found in the references (Kaska et al., 1998, 2006).

1.3.3.4.1. Monitoring of Beach erosion and Coastal development

Coastal development is largely the result of recreational/tourist activity. It is associated with the presence of hotel resorts and other tourism related constructions such as restaurants, bars, houses and related businesses, typically built along the beach, impacting an originally flexible and adjustable coastal system. There are many examples of these developments on the nesting beaches of sea turtles in the Mediterranean and all such activities and changes in the nesting habitat should be monitored.

Beach erosion and beach armouring may also be recorded, as this very much relates to changes in the ecological conditions of the nests and the development of embryos and hatchlings.

Coastal development is also associated with the activities that have an impact on sea turtle nesting activity. Driving on the beach and the use of heavy machinery for beach cleaning purposes are common practices and are responsible for alterations in sand characteristics and the destruction of turtle egg clutches.

Water sports, a leisure activity closely linked with high tourist activity, can lead to collisions between turtles and speed boats, especially close to nesting areas where turtle density is high. Such recreational activities and their potential impact on sea turtles should be recorded and necessary precautions and mitigation measures need to be taken into account.

Coastal development can be easily monitored during beach monitoring studies. The nesting beach can be photographed at the beginning, middle and end of the nesting season and GPS coordinates recorded. This procedure can be repeated each year. Optionally, satellite images from previous years can be used for comparison. Free images are available from different sources (e.g. https://earthengine.google.com/timelapse/).

1.3.4. Growth, Age at Sexual Maturity and Survival

Different aging methods result in the similar estimation of Age at Sexual Maturity (ASM), ranging between 14.9-18.6 years for small nesters of 66 cm CCL and 26.3-34.9 years for larger reproductive females of 84.7 cm CCL (see references in Casale et al., 2018). The mean size of female loggerhead turtles nesting in the Mediterranean is 79.1 cm CCL and males appear to reach maturity at a similar size (Casale et al., 2005; Casale et al., 2014). The average ASM for the Mediterranean loggerhead population was estimated at 25 years (range: 21-34 yrs) from the mean values of the eight age-at-length relationships obtained by the above studies, applied to a size at maturity of 80 cm CCL (Casale & Heppell, 2016).

Mediterranean loggerheads appear to reach 28 cm CCL at about 3.5 years old, with the growth rates ranging from 11.8 cm year^{-1} in the first months of life to 3.6 cm year^{-1} at the age of 2.5-3.5 years, similar to that of Atlantic loggerhead turtles (Casale et al., 2009). Broderick et al. (2003) reported growth rates of 0.36 cm year^{-1} for loggerhead females nesting in Cyprus.

Based on capture-mark-recapture data, the annual survival probability of loggerheads of 25-88 cm CCL was estimated at 0.73 and this was considered to be underestimated by at least 0.1 because of tag loss (Casale et al., 2007b). The annual survival probabilities of large juveniles at four different foraging areas were estimated through a catch curve analysis, resulting in values ranging 0.71-0.86 depending on the area (Casale et al., 2015). These values were considered to be lower than expected from a healthy population and are possibly due to anthropogenic mortality such as bycatch, especially in some areas like the south Adriatic (Casale et al., 2015).

For green turtles, the current information on growth rates is limited to adult females showing a slow growth of 0.11 cm yr^{-1} CCL (Broderick et al., 2003).

Oceanic nursery areas for post-hatchling and small juvenile turtles (< 40 cm CCL) are largely unknown in the Mediterranean. Loggerhead turtles, especially juveniles, can be found in virtually all oceanic areas within the Mediterranean. Their distribution is fundamentally driven by the circulation system of the Mediterranean as indicated by genetics (Carreras et al., 2006), telemetry (Revelles et al., 2007) and flipper tagging (Casale et al., 2007a; Revelles et al., 2008). Identifying the most frequented areas is not a simple task and at present the best insights are provided by interaction with fisheries. Turtles in the oceanic zones belong to at least three different Regional Management Units (RMUs) (Wallace et al., 2010): the Mediterranean, the Northwest Atlantic and, to a lesser extent, the Northeast Atlantic (Clusa et al., 2014). Juveniles from Atlantic RMUs enter the Mediterranean through the Straits of Gibraltar and mainly distribute across the south of the western basin following the less saline waters from the Atlantic (Millot, 2005). They can also be found in other regions of the Mediterranean, but at much lower proportions (Clusa et al., 2014). Juveniles from the Mediterranean RMU can be found throughout the basin, although their relative proportion is greater in the eastern, central and north-western Mediterranean (Clusa et al., 2014).

Adult sea turtles in the Mediterranean are primarily found in neritic areas, and also on the nesting beaches. Loggerhead turtles can be encountered at pelagic areas, but priority should be given to the aggregation areas in neritic habitats, taking time, budget, and human resources into account. Population demographic parameters need to be collected by conducting in-water studies for both species, especially for juveniles and sub-adults.

1.3.5. Data can be collected from Fishermen-Fisheries Interaction

There is a large body of data on turtle bycatch in the Mediterranean, which has recently been reviewed, showing that the level of information available is not equal across countries or sub-regions (Casale, 2011). This review estimated more than 132,000 captures and 44,000 deaths in the Mediterranean annually, from all gear combined. The resulting ranking order of different fishing gears for the number of captures per year was: pelagic longline, bottom trawl, set net and demersal longline. For fatalities, the ranked order was: pelagic longline, set net, bottom trawl and demersal longline.

Small-scale fleets (SSF), polyvalent vessels of up to 12 m in length, are the dominant fishery segment and account for 80 percent of the total vessels in the Mediterranean and Black Sea (FAO 2016). Sea turtles are at high risk from SSF, possibly due to the long soak durations of gear (Carreras et al., 2004; Echwikhi et al., 2010, 2012; Coelho et al., 2013) and this fishery may be responsible for most of the fishing-induced mortality in the Mediterranean (Casale, 2011).

Bottom trawlers cause death by drowning and mitigation measures are represented, among others, by the modification of the gear (turtle excluder device or TED) to enable any captured turtle to exit the net (FAO, 2009; Lucchetti et al., 2016) and by keeping comatose (i.e. semi-drowned) turtles on-board until they recover (Gerosa & Aureggi, 2001; FAO, 2009). However, decompression sickness may represent an additional and overlooked problem (García-Párraga et al., 2014). Pelagic longlines generally cause death after release, as result of internal damage caused by the line and secondarily by the hook (Casale et al., 2008; Parga, 2012; Alvarez de Quevedo et al., 2013). Mitigation measures are represented, among others, by using larger hooks (e.g., circle hooks) (Piovano et al., 2012; Gilman & Huang, 2017), which decrease the catch rate and by removing the gear (especially the line) from the turtle before releasing it (Gerosa & Aureggi, 2001; FAO, 2009). Set nets cause death by drowning, with very high mortality rates due to the long time the net is left in the water (Echwikhi et al., 2012) and the only mitigation measure available at present it the illumination of the net, so that turtles can see and avoid it (Ortiz et al., 2016).

The highest catch rates in the Mediterranean have been observed off the coast of Tunisia, in the Adriatic Sea and in the easternmost part of the Levantine basin, off Turkey, Syria and Egypt (Casale, 2011; Casale et al., 2012b). A regional bycatch project (supported by the MAVA foundation) should be established to update bycatch figures.

2. MONITORING METHODS

The monitoring of sea turtles can be performed by:

a) counting the number of nests during the nesting period and monitoring nest parameters
b) collecting stranded turtles and obtaining information from collected tissues
c) in-water capture-mark-recapture studies for population distribution
d) boat and aerial surveys can also be used for the beach monitoring and in-water monitoring of sea turtles

To monitor the distributional range, the population abundance and the demographic characteristics of sea turtles, two monitoring methods can be applied:

- beach monitoring: ground based or aerial monitoring
- in-water monitoring: boat based or aerial monitoring

Table 7. Data to be collected, data collection tools, and relevant common indicator.

Common Indicator	**Nesting Beach Monitoring**		**Marine Habitat Monitoring**	
CI3 Distribution range	*Implementation/ Tools*	*Data collected*	*Implementation/ Tools*	*Data collected*
	Beach foot patrol	Yearly number of nests and tracks; nesting success; spatial and temporal distribution of nests	Boat surveys	Number of individuals; size classes; species distribution; habitat use
	UAV or plane surveys	Number of tracks, and identify nests if possible	UAV or plane surveys	Number of individuals; size classes; species distribution
	Satellite-GPS tracking turtles	Migratory corridors, cutch frequency, inter-nesting habitats, feeding grounds	Satellite-GPS tracking turtles	Migratory corridors; wintering areas; nesting grounds; habitat use
	Sand, nest, and sea water temperature monitoring	Sex ratio trends; suitable nesting beaches; nesting periodicity	Fisheries bycatch data	Sex ratio, maturity, distribution of species, size classes; number of individuals
	Stranded turtle network	Spatial and temporal distribution and age classes of turtles	Stranded turtle network	Spatial and temporal distribution and age classes of turtles
	Stable Isotope Analysis	Habitat use; estimating origin of feeding ground;	Stable Isotope Analysis	Habitat use
	Monitoring potential nesting grounds	Yearly number of sporadic nest counts		
	Photo ID, flipper tag, PIT tag, genetic tag	Number of individuals; multiple paternity; haplotype diversity		
CI4 Population Abundance	Beach foot patrol	Yearly number of nests counts and the number of nesting females	Boat surveys	Number of individuals; size classes; species distribution
	Photo ID, flipper tag, PIT tag, genetic tag	Number of individuals; multiple paternity; haplotype diversity	Genetic sampling	Mix stock analyses; genetic diversity (mitochondrial and nuclear DNA)
	Monitoring potential nesting grounds	Yearly number of sporadic nest counts	Fisheries bycatch data	Sex ratio, maturity, distribution of species, size classes;

				number of individuals
			UAV or plane surveys	Number of individuals; size classes; species distribution; habitat use
			Stranded turtle network	Spatial and temporal distribution and age classes of turtles
CI5 Population Demographics	Beach patrol	Hatching and emergence success; predation rate; hatchling sex ratio	Boat surveys	Number of individuals; size classes; species distribution; habitat use
	Photo ID, flipper tag, PIT tag, genetic tag	Number of individuals; multiple paternity; haplotype diversity	CMR studies	Age and size classes, sexing, maturity, health status
	Stranded turtle network	Aging dead turtles through skeletochronology	Genetic sampling	Mix stock analyses; genetic diversity (mitochondrial and nuclear DNA)
			Stranded turtle network	Spatial and temporal distribution and age classes of turtles
			Fisheries bycatch data	Sex ratio, maturity, distribution of species, size classes

Both methodologies can be applied for the Loggerhead turtle as well as the Green turtle. Selecting the most appropriate monitoring method depends on the budget, equipment and personnel available. Beach monitoring should be established on all known nesting beaches, on daily basis, during the nesting period. Potential nesting sites may also be monitored once or twice a week. The monitoring of beaches allows for counting the emergence of adult female turtles, their clutches, and the number of hatchlings. Therefore, estimates for breeding populations can be calculated. For ground-based monitoring, the number of people working in the field depends on the size of the beach, while the equipment can easily be acquired on a low budget. For instance, for daily foot patrols, at least three (2-8) people should be considered for a five km nesting beach.

The monitoring of in-water populations requires more expensive equipment, such as boat, entanglement net, or Unmanned Aerial Vehicles (UAVs).

2.1. Time and Area

Sea turtles are a highly migratory species. They can be found in different habitats at different times of the year. Therefore, the demography and sex ratio of the population changes temporally throughout the year. Breeding, foraging and overwintering areas are the main ones to be monitored.

2.1.1. Breeding Area

2.1.1.1. Nesting Female Population

Nest counts, the direct observation of nesting females, and reproductive outputs are observed during the nesting season. The monitoring of nesting beaches starts at the beginning of May and continues until the end of September, every year.

2.1.1.2. Operational Sex Ratio

Operational sex ratio is the proportion of ready to mate individuals from both sexes. This requires the direct sampling of individuals from the sea. In the Mediterranean, mating mainly occurs during April and May. Therefore, monitoring activity should start in April and continue until the end of May and it should be conducted every year.

The monitoring of the operational sex ratio before April and after May should be avoided, as individuals captured during these periods may represent different populations and the results can be misleading.

2.1.2. Foraging and Overwintering Areas

Monitoring sea turtles at foraging and overwintering sites can be conducted annually and throughout the year. Loggerhead turtles can be found throughout the Mediterranean, especially in bays and estuaries. Green turtles can be found in the eastern Mediterranean and are rare in western locations. The best period for monitoring foraging and overwintering areas is during the months of September and October, as the turtles will have completed their post-nesting migration.

2.2. Samples and Data to be Collected from Sea Turtles

Implementation and/or sampling	**Data to be collected**	**Monitoring methodology**		
		Beach Monitoring	**In-water Surveys**	**Stranding**
Morphometric measurements	• Size class • Age at Sexual Maturity	X	X	X
Tagging *Metal tags* *Plastic tags* *PIT tags* *Photo ID*	• Population size estimates • Inter-nesting period • Migration route	X	X	
Sampling skin	• Genetic analysis • Stable isotope analysis • Trace element analysis • Heavy metal analysis	X	X	X
Sampling scute	• Stable isotope analysis • Trace element analysis • Heavy metal analysis	X	X	X
Sampling blood	• Genetic analysis • Blood biochemistry and health parameters • Sexing juveniles	X	X	

	• Blood cell physiology • Stable isotope Analysis • Trace element analysis • Heavy metal analysis			
Tissue sampling from internal organs and muscles	• Histologic investigation • Genetic analysis • Heavy metal analysis • Marine litter ingestion			X
Parasyte – Epibiont	• Health status • Stable isotope	X	X	X

2.2.1. Size measurement of individuals and Tagging

Regardless of monitoring methodology, measuring carapace length is an essential tool for identifying the age class of sea turtles.

Adult body size varies greatly among different nesting sites for both species. One of the most distinctive characteristics of Mediterranean loggerhead turtles is a smaller adult female size in comparison with other populations worldwide (Tiwari & Bjorndal, 2000; Kamezaki, 2003). Some loggerhead males start to develop an elongated tail at size >60 cm CCL (Bolten, 1999) and a clear dichotomy in this trait is evident in the population in the >75 cm size class CCL (Casale et al., 2005; Casale et al., 2014). For Straight-line Carapace Length (SCL), 70 cm is usually accepted as a mature female. This type of information can only be obtained by the measurement of individuals.

Sea turtle measurement techniques, as explained by Bolten (1999), are frequently used. The measurement of carapace length is an important parameter for identifying size classes. The most common measurements are given below:

- Straight carapace length (SCL): A calliper is used to measure straight length. Three types of measurements are available for SCL:

(i) SCL_{min}: measured from the anterior point at midline (nuchal scute) to the posterior notch at midline between the supracaudals

(ii) SCL_{n-t}: measured from the anterior point at midline (nuchal scute) to the posterior tip of the supracaudals.

(iii) SCL_{max}: measured from the anterior edge of the carapace to the posterior tip of the supracaudals.

- Curved carapace length: A tape measure is used to measure straight length. Three type of measurements are available for CCL:

(i) CCL_{min}: measured from the anterior point at midline (nuchal scute) to the posterior notch at midline between the supracaudals

(ii) CCL_{n-t}: measured from the anterior point at midline (nuchal scute) to the posterior tip of the supracaudals.

(iii) CCL_{max}: measured from the anterior edge of the carapace to the posterior tip of the supracaudals.

- Straight carapace width (SCW): A calliper is used to measure the straight width of the carapace. SCW is measured at the widest point and there is no anatomical reference point for the measurement.

- Curved carapace with (CCW): A tape measure is used to measure straight width of the carapace. As in SCW, CCW is measured at the widest point and there is no anatomical reference point for the measurement.

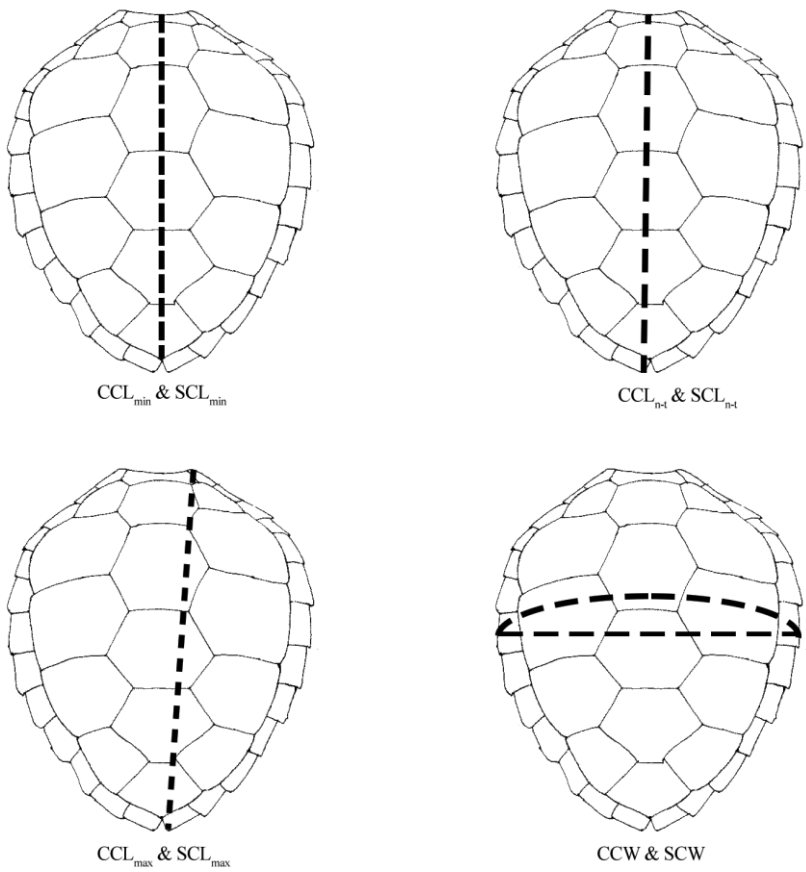

Figure 2. Morphometric measurements of carapace. (For abbreviations see the above text)

Tagging is an important tool for monitoring sea turtle populations, as it allows the identification of each turtle as an individual. Each size class of sea turtle, apart from hatchlings, can be tagged. Different types of external tags are available; the most common are Monel, Inconel and Plastic flipper tags. These tags can be found in various sizes and should be selected accordingly to the size of the turtle. A range of tag models can be found at https://nationalband.com/.

The advantages of these tags are:

- Visual identification is possible without additional equipment or device by different researchers, fishermen or any person who encounters the turtle. Tag returns are important for monitoring projects
- Cheaper in comparison with other methods.

The disadvantages of these tags are:

- High loss rates, especially when the turtle is not properly tagged
- External tags may cause entanglement in fishing nets or marine garbage

Passive Integrated Transponders (PIT tag) are also used in monitoring projects. This is an invasive technique that can be applied with a gun or a needle applicator. Sea turtles are tagged with a very small microprocessor. Although the PIT tag remains in the turtle's tissue and may have a low tag loss rate, these tags are not visually identifiable, and an electronic reader is required. Furthermore, PIT tags are more expensive than flipper tags.

Photo-identification: Photo identification is an alternative tagging method that is becoming increasingly popular. The methodology is minimally invasive, as it is a technique that basically depends on photographing an individual's scales, creating photo database, and evaluating database photos. Computer programmes for photo-identification are available. The lateral scale patterns of turtles are commonly used. To obtain the best results, photographs should be taken from the same distance and angle for each individual.

Required Equipment

Measuring the Size

- Notebook
- Pencil
- 150 cm long calliper
- 150 cm long tape measure

Tagging

- Monel, Inconel, or plastic flipper tags
- Tagging Pliers (different pliers for each type of the tags)
- PIT tags
- PIT tag needle applicator or applicator gun
- Electronic PIT tag reader
- Camera

2.2.2. Skin and Scute Sampling

Carefully clean the sampling area prior to the procedure. First, gently clean the sampling area to remove all possible epibionts and algae and rinse the area with water. Next, clean with ethanol or another disinfection agent. Using a 6 mm biopsy punch is an easy way to take skin samples. If a scalpel is being used, the turtle should first be restrained and immobilized. After stabilizing, use forceps to facilitate sampling. The biopsy should be no deeper than 0.5 mm. This will prevent bleeding. After sampling, clean the area with betadine to prevent any bacterial infection. Tissue samples should be placed in ethanol (70% or 96%). Use disposable single-use sampling materials and gloves. Using the same sampling materials – such as a biopsy punch or scalpel for different turtles – may transfer DNA from one sample to another. Place the samples in cryovials or Teflon bags and store, frozen to at least -20°C, until analysis.

There are two preferred methods for collecting scute samples. The first is by cutting a small piece of keratin with a biopsy punch or scalpel, and second is by shaving. If the turtle is large, use a biopsy punch or scalpel to sample the scute, as this enables different layers of keratin tissue to be collected.

After cleaning the area of algae, sand and any other materials, the top layer can be gently shaved then rinsed with distilled water, if possible. A 1X1 cm scute sample is usually sufficient for analysis. Place the samples in cryovials or Teflon bags and store, frozen to at least -20°C, until analysis.

If sampling is taken from a juvenile turtle, it can be collected via shaving the scute. The keratin layer is very thin, especially with green turtles. Clean and rinse the sampling area, then start shaving an entire scute by using a knife (5th ventral scute is suitable for this procedure). Approximately 2.00 mm of the keratin should be shaved. Using a wind shield (e.g. umbrella) whilst shaving is beneficial.

2.2.3. Blood Sampling

Blood is widely used for scientific purposes, such as:

- Diagnosing a turtle's health status
- Physiologic studies (blood cells, hormones, antibodies, etc.)
- Blood biochemistry studies (electrolytes, blood enzymes, proteins)
- Sex identification (hormones and enzymes)
- Stable isotope analyses
- Genetic analyses

Whole blood tissue comprises two main parts: blood cells and plasma. A study can therefore be made using whole blood, blood cells (haematocrit), or plasma. In each case, a sufficient amount of blood should be collected and stored. If the blood sample is not properly collected and/or is incorrectly stored, the results will not be reliable.

Blood sampling should be completed as soon as possible after the capture of the animals; ideally, within 5 minutes of capture and a maximum of 15 minutes. A sea turtle's dorsal cervical sinus is an easily accessible location for taking blood samples. The turtle should be restrained in stable position. The best position is to lift the turtle's back, as this will help to fill the cervical sinus with blood. Gently pull the head forward and downward to stretch the neck.

Once the neck is stretched, locate its midpoint. Move 1 cm. towards the nuchal scute, a suitable area for blood collection. Do not insert the needle into the median line of the neck, as this could strike the vertebral column. When the neck is stretched, two tendons become visible. The needle can be inserted by these tendons, at the lateral sides. Insert the needle vertically. Suction should start after passing the integument. Carefully continue to insert the needle downward, using a small amount of suction until the blood starts to flow. On seeing the blood, maintain the needle in a stable position until sufficient blood is collected.

Required equipment

- 21g Needle and Syringe/Vacutainer
- Heparinized blood tubes
- Centrifuge (for separating blood cells from the plasma)
- Vials and cryo tubes
- Ice box (for transportation)
- Gloves
- An antiseptic (e.g. ethanol)

2.3. Beach Monitoring

Beach monitoring should be conducted at night or during morning patrols. Night patrols permit encounters with nesting females, while finding nests at night helps them to be protected from predation, inundation risk, or poaching. Night patrols begin after sunset and may continue until morning. Morning beach surveys start at dawn.

Required Equipment

- Notebook
- Pencil
- Measure tape (30 m or longer)
- GPS
- Headlamp with red-light
- Camera (optional)

To determine turtle activities, potential nesting sites should be monitored every two weeks during the summer period. Beaches identified as nesting areas should be monitored every 1-3 days for nest/track counts. During these visits stranded turtles can also be recorded and the necessary samplings conducted.

2.3.1. Beach Monitoring during nesting season

Existing and potential nesting beaches should be monitored during the nesting season. Ground-based surveys with a hand-held GPS should be used to map the sea turtle nesting beaches.

- All equipment must be ready prior to beach monitoring.
- At night, only red-lights should be used on beaches; ideally, patrol teams should be silent, and any sounds should be minimal.
- On patrols, avoid large numbers of people.
- To avoid covering sea turtle tracks, patrol teams should walk on wet sand in the ebb tide. Once a track is found, only one person should follow the track, notifying the rest of the team if a female sea turtle is found.
- If a turtle is found, the group should sit quietly, waiting until it finishes laying its eggs and starts to cover the nest.
- It will save time if the location can be marked at this stage.
- The sea turtle should be tagged and measured as soon as it finishes laying. Once the turtle is tagged, it should also be recorded.
- Tissue samples should be collected after tagging. If sensitive samples are to be taken, such as blood, these should be collected first.
- Minimal light should be used to record data, to avoid distracting the female and affecting the nesting activity.
- The location of the nest should be recorded using physical measurements. To obtain three-point positioning, measure the distance from the shore line and also from at least 2 permanent points at the back of the beach. Record the GPS coordinates.
- The nest should be covered with a grid to protect it from predation (eggs dug up by animals searching the beach for food).
- All turtle tracks should be erased, so subsequent teams can clearly see new tracks and are not distracted by tracks and nests that have already been logged.
- The presence of predators (dogs, cats, ferrets, seabirds, foxes etc.) on the beach can be recorded by direct observation and the documentation of tracks. If a predation occurs, it should be recorded immediately. In such cases the actions to be taken are given below:
- The predator should be identified. Egg shells scattered around the nest should be collected and counted to establish how many eggs have been damaged as a result of the predation.
- In cases of infestation in the scattered eggs, specimens (adults, pupae, larvae) should be collected for further examination in the laboratory
- The damaged eggs should be removed from the beach.
- The centre of the predated and distorted nest should be located and opened
- Carefully search for intact (undamaged) eggs.

- In cases of completely ruined nests where intact eggs are observed, excavate a new nest close to the existing one and carefully relocate the undamaged eggs.
- The eggs should be kept in the same position (for transporting over long distances, mark the top of the eggs with marker pen) to avoid them being affected by vibration, rotation or temperature changes. The number of the intact eggs and the GPS coordinates of the new nest should also be logged.
- For every measurement location or sampling collection point, the GPS position should be recorded, and all information should be added to the GIS database

Aerial surveys are also an effective way of monitoring of nesting beaches; when the nesting beach is in a remote area, the beach is long, or human resources and equipment are insufficient. Ariel surveys by UAV or plane may be used for counting sea turtle tracks and nests. Surveys can be conducted daily, on alternate days, or on a weekly basis.

2.3.2. Beach Monitoring during the hatching season

Data collected during the nesting season is used to estimate the hatching period. This will be confirmed by physical evidence and the observation of tiny tracks leaving the nest towards the sea.
The hatching period usually occurs between 45 and 70 days after the first nesting date. Nests that have reached the 40 days incubation period should be monitored.
Nest excavations should be conducted 4 days after spotting the first tracks and the following data should be recorded:

a) Live hatchlings
b) Dead hatchlings
c) Yolk sacks still attached
d) Half developed eggs
e) Unfertilized eggs
f) Empty shells

2.3.3. Hatched Nest Excavation

Nest excavations are essential for saving hatchlings that are unable to exit the nest because they are not strong enough or due to the nest being closed by an external factor.
During a nest excavation, information is recorded about healthy hatchlings, unfertilized eggs, dead embryos, empty shells and live hatchlings that could not exit the nest.
Egg shells found in the nest are recorded as empty shells, and eggs with dead embryos inside are recorded as dead embryos. However, the detection of dead embryos early in life can be difficult.
Data collected during the nest excavation are given below:

- Early Stage Embryo: An embryo that is smaller than 1 cm. The embryo may have died a few days after egg laying. For this reason, it is difficult to distinguish an early stage embryo from an unfertilized egg.
- When the egg is opened in such cases, a blood clot should be observed, and the egg yolk should be still be attached to the shell. Also, the part of the outer shell should be examined for the clarification of the whitening calcium layer, due to the breathing of the embryo. Furthermore, all or part of the egg colour will be white. If the egg has these characteristics, it is called early stage embryo.

i. Middle Stage Embryo: These are embryos of between 1 and 2 cm.
ii. Late Stage Embryo: These are embryos larger than 2 cm.
iii. Dead Hatchling in the Nest: These hatchings are found in the nest during the excavation process.

iv. Live Hatchling Outside the Nest: These hatchlings are found during field work, or their presence is determined by the tracks they leave.
v. Dead Hatchling Outside the Nest: These hatchlings are detected during field work on the beach, by their traces, which do not reach the sea.
vi. Unfertilized Eggs: Eggs in which the embryo failed to develop. These eggs are yellowish-brown or greyish in colour and show none of the above characteristics.
vii. Empty Shells: Eggs shells left behind by the hatchling after emerging.
viii. Alive Hatchlings in the Nest: Living hatchlings found in the nest during the excavation process.

The timing of nest excavations for control is variable. The first nests of the season (April, May and early June) usually have a longer incubation period and it takes longer for hatching to commence in these nests with incubation lasting up to 70 days. The hatchlings that belong to these earliest nests may take 8-10 days to hatch.

Nests from the middle of the season have shorter incubation period, when 45 days is sufficient for the incubation process. The complete hatching process may take only a few days, although in some cases it can last as long as 6-7 days. Excavation for these nests should be made 5-6 days after the first hatching. During excavation, live hatchlings that have reached the sea; unfertilized eggs; dead embryos; dead hatchlings; empty shells and living hatchlings still in the nest, should be recorded.

2.3.3.1. Calculation of Hatching and Incubation Period

Usually, the surface of the nest collapses 2-3 days before the hatching begins and the egg crumples as the hatchlings begin to emerge, allowing sand to enter. This movement opens a route through which the hatchling can emerge from the nest. At night, the temperature of the sand decreases and the hatchlings start scrambling to the surface. Most of the hatchlings exit the nest on the first night and the rest during the next few days. The hatching process is usually completed within a week.

The incubation period is from the nesting date to the date of the first emergence of hatchlings and is measured on a day-by-day basis.

2.3.3.2. Calculation of Hatching Success

- Hatching Success = (Empty Egg Shells) / (Total Number Eggs) X 100
- Total Number of Eggs = EES + UE + DE + PE
- EES: Number of empty egg shells; UE: Number of unfertilized eggs; DE: Number of dead embryos; PE: Number of predated eggs

2.3.3.3. Sand, Nest, Sea Surface Temperature

It is recommended that sand, nest and sea surface temperatures are monitored to track the effect of climate change. The temperature of these environments is a useful gauge for assessing different parameters.

Sand Temperature	**Sea Surface Temperature**	**Nest Temperature**
Affects nest temperatures	Affects nest temperatures (see Girondot and Kaska, 2015)	Sex ratio estimates
Temporal and spatial temperature changes in different beach sections	Breeding periodicity of adults	Assessing hatching success

The use of data loggers that record temperature is a common and simple way for monitoring sand and nest temperatures. Sea surface temperature may be recorded, or the data can be requested from national meteorological organisations.

Monitoring sand temperature: Data loggers are placed at specific intervals on the nesting beach. For most sea turtle nesting sites, 1 km intervals between each data logger is preferred, buried at a depth of 50 cm, although this depends on the condition of the beach. When placing/planning devices, attention should also be paid to the following:

- Devices should not be placed in the inundation zone.
- If possible, devices should be placed in different zones within the location (e.g. nesting zone, vegetation zone).
- Devices should be placed by the second week of April and collected at the end of September.
- The beach structure is likely to be affected by natural phenomenon; for instance, winds, waves, and inundation. It is therefore advisable to take precautions, such as fixing the devices or covering them with grids.
- Take GPS coordinates of the device locations.

Required equipment

- Data loggers
- GPS
- An interface programme (to programme devices and download data)

2.4. Monitoring of Abundance of In-Water Population

2.4.1. Boat Survey

In-water population monitoring is used to estimate the population size, abundance, and sex ratio of a population in a particular area. It is also very useful for collecting biological samples.
A research area can be a breeding, feeding, overwintering ground or a mixture of these three areas. This means that different populations can be found in an area. Sea turtles are a migratory species, so the timing of the study is important and should be selected carefully and a standardized methodology should be followed.

Boat based survey: capture-mark-recapture (CMR) method
Two common methods are used for in-water surveys. First, a capture net is set in the sampling area. Second, the turtles are captured using the rodeo technique.

(i) In the case of a large study area with low visibility and deeper water, a capture net is preferable.
(ii) The mesh size of the net should be large to avoid the by-catch of other marine animals but small enough to capture turtles.
(iii) The mesh size of the net can be from 10 to 15 cm. Once the net is set, it should be monitored regularly from a boat.
(iv) If the team is sufficiently large and the visibility is high, it is best to swim to the net for this study.
(v) When a turtle becomes entangled in the net, it should be removed and transferred to the boat for measurements and sample collection.
(vi) The turtle should remain on the boat until the net is collected and then released into the sea.
(vii) This study can be used to estimate the size and sex ratio of the population.
(viii) The rodeo technique requires smaller team and can be used in small areas and in shallow waters with high visibility.
(ix) When a sea turtle is spotted from the boat, a swimmer dives and captures the turtle.

(x) The sea turtle is then measured and biological samples are collected.

Required Equipment

- A Boat
- Entanglement net
- Measurement equipment
- Tagging equipment
- Balance
- Snorkel
- Mask
- Fins
- Ultrasonic-type depth meter
- GPS
- A minimum of five crew members, which can be increased according to type of study, area, and budget

2.4.2. Satellite Tracking

A satellite telemetry of adult sea turtles is required for identifying the foraging grounds used by the adults of each population. This technique can also be used to assess the surface time of turtles at foraging grounds. A parameter is necessary to derive absolute population estimates for aerial surveys.

2.4.2.1. Application of satellite tags and data loggers

Satellite tracking is one of the most commonly used techniques for tracking sea turtles, as it can determine migratory corridors, feeding and overwintering areas. It also gives precise information on the localisation of the animal. However, as the cost of the tracking devices is high, this may limit the number of turtles that can be tracked.

The Argos tracking system is the most commonly used, but the Iridium satellite device has become a new option in recent years. The systems work in similar way, and a common methodology is used for attaching transmitters to turtles.

Before attaching the transmitter to the sea turtle it should be checked using a small receiver device. If the transmitter is emitting signals, turn the receiver device off and prepare the turtle for the attachment.

The turtle can be stabilised in a large tank (1m X 1.5 m). The transmitter is normally attached on the second vertebral scutes. The attachment area on the carapace should be cleaned of epibionts, then rubbed with sand paper until smooth. Carefully remove any dust and swab the area with acetone, before leaving it to dry for a few minutes.

Use a strong glue, such as marine epoxy, to attach the device. Depending on the type of glue being used, it can be mixed prior to application, or on the carapace itself. The glue is also applied to the device but avoid getting it on important parts, such as the magnet connection point or sea water switches. After completing the attachment, leave the sea turtle in the open air until the glue is completely dry. Then it can be released into the sea.

Double check! Make sure the device is switched on before releasing the turtle. Forgetting to check that the transmitter is operational before the release is a common mistake.

Required Equipment

- Satellite transmitter tags (order at least two months before they are needed)

- Container for handling turtle (100 X 150 cm)
- Sandpaper
- Acetone
- Glue (marine epoxy resin)
- Magnets (to switch on and off the tags)

2.4.3. Aerial Surveys and use of UAV

Aerial surveys are the best method for determining the abundance of turtles at sea and detecting changes in population, before they translate into changes in nest counts.

Aerial surveys necessitate information about time spent on the surface, in order to produce absolute estimates of turtle abundance. Drones, for monitoring nesting activities and making individual counts of sea turtles swimming on the surface, are becoming popular in recent years.

Aerial surveys should be conducted every five years at each major foraging ground (Alboran Sea, Balearic Sea, Algerian Basin, Tyrrhenian Sea, Libyan Sea, Adriatic Sea, Aegean Sea, the southern coast of Turkey and the Levantine Sea).

Unmanned aerial vehicles (UAVs) or drones are increasingly being adapted for gathering data, at previously unprecedented spatial and temporal resolutions, in diverse geographic locations. This easily available, low-cost tool is improving existing research methods and enabling novel approaches in sea turtle ecology and conservation. For studies on turtle nesting, sea distribution and behaviour surveys, UAVs can reduce costs and field time, while improving safety, as well as data quality and quantity, over existing methods. They are also expanding into new avenues, such as the surveillance of illegal take (See Rees et al., 2018 for further information).

However, there are some limitations on the use of UAVs:

(i) They require a trained pilot
(ii) The battery life of most UAV's is less than 30 min. Therefore, flight time and the monitoring area should be carefully determined before starting the study.
(iii) Meteorological conditions (strong winds, light, etc.)
(iv) Legal limitations (no-flight zones, necessary licences and permissions)
(v) Ethical implications (privacy, effects on animals etc.)

Required Equipment

- UAV (DJI drones are the most common for sea turtle research)
- Trained UAV pilot
- Tablet, computer
- Remote control device
- Replacement batteries

2.4.3.1. Monitoring Remote Nesting Beaches

A UAV can be used for the regular monitoring of remote beaches with low nesting density, especially when the beach is inaccessible. This saves time and gives precise information about sea turtle nesting activities.

2.4.3.2. In-Water Observations

UAVs are very useful tools for monitoring in-water populations. They can be used to determine the density and distribution of sea turtles in foraging areas, as well as investigating their behaviour, monitoring and mapping habitats.

2.4.4. Genetic structuring

Molecular genetic techniques are widely used and there are several non-invasive sampling methods. Although these look simple enough, they require close attention during sampling, due to the possible contamination of DNA from different individuals. Genetic samples can be collected from adult females, hatchlings and dead embryos.

Blood and skin are the two most common tissues used for collecting genetic samples. Blood collection is described above. A tissue biopsy from skin is straightforward: tissues are collected from the front or (preferably) the rear flipper using a biopsy punch. If no biopsy punch is available, use a scalpel. A skin sample of 1.5 to 2.0 cm is adequate for genetic analyses. To prevent bleeding the biopsy should be no deeper than 0.5 mm.

After sampling, clean the area with betadine to prevent any bacterial infection. Place the tissue sample in 70% ethanol. Always use single-use disposable sampling materials and gloves. If the same sampling materials are used, such as biopsy punch or scalpel, for different turtles, DNA may be transferred from one sample to another.

For genetic analyses, take a small amount of muscle from a dead turtle during necropsy. It is best to collect the same tissue for each research study, if possible.

Cheek swabs and carapace scrubbing are other sampling methods. A cheek swab is not ideal, as the mouth of the turtle must be kept open during sampling.

When collecting samples for a stable isotope from the carapace, carapace scrubbing can be used. When scrapping a carapace, the white epidermal tissue can be seen on the inner part of the carapace sample. Rinse the carapace sample and let it air-dry for a short period. It is easy to remove the epidermal tissue and store the sample in ethanol.

Available information is based on the use of mitochondrial haplotypes and nuclear microsatellites. This allows the individual assignment of loggerhead and green turtles to major nesting areas in the Atlantic (Carreras et al., 2011, 2014).

Genetic structuring on nesting beaches and in foraging grounds is better determined by using genetic analyses together with other nesting information, such as remigration interval and clutch frequency through female fingerprinting. This helps to understand the genetic contribution made by nesting beaches to foraging grounds.

2.4.5. Monitoring stranding

Most research on sea turtles has traditionally been conducted on nesting beaches, even though they spend most of the time in the ocean. The available information suggests that turtles do not distribute homogenously within the sub-basins (Clusa et al., 2014) and that some key parameters, such adult body size and fecundity, vary between females foraging in different sub-basins, although they nest on the same beach (Zbdinen et al., 2011; Cardona et al., 2014). Therefore, detailed information about adult habitat use is critical, albeit some for major nesting beaches is still missing.

Stranded turtles are a good data source for collecting various data about sea turtle biology and possible threats. The following information can be collected from stranded turtles:

- The spatio-temporal distribution of turtles
- Tissue sampling for genetic and stable isotope analyses
- Bone sampling for skeletochronology
- Size classes
- Sex
- Threats (cause of deaths)
- Marine pollution (marine litter ingestion; monitoring organic and chemical pollutants in the marine environment).

Common protocols are available for data collection from stranded turtles. For example, a detailed protocol for collecting data from stranded turtles, in order to monitor marine litter ingestion, was prepared by the INDICIT consortium. This can be found at their project website https://indicit-europa.eu/indicit-documents/

2.4.5.1. The Monitoring of Pollution and Pollutants

Sea turtles can ingest or become entangled by anthropogenic debris. In contrast to ingestion, entanglement has been reported as an important cause of stranding in the Mediterranean (Tomás et al., 2008; Casale et al., 2010). Studies on marine debris ingestion by sea turtles in the Mediterranean have been reviewed by Casale et al. (2016). It shows that the occurrence of marine debris varies among studies, with the highest occurrence (80%) reported from turtles caught by pelagic longlines in the central Mediterranean (Casale et al., 2016). Investigations into plastic ingestion can be made using the necropsies of dead turtles but contamination from the environment during the necropsy should be avoided.

Before removing the GI tract, tie the anterior part of the oesophagus. Then, tie it above cardiac sphincter and at the beginning of intestine (after the pyloric sphincter). Finally, tie the end of the intestine. In this way the contents of the different GI tract sections will not become mixed.

The working space should be cleared before an investigation of the GI tract for possible contamination. Cut each section apart, then measure the weight (and the volume, if required) of the sections (oesophagus, stomach, intestine).

Start by cutting each section separately and placing them in a sieve with a mesh size of 1 mm under running water. Collect each foreign object from the contents of each section and place in a container with 50% ethanol. Collect organic materials for diet studies and keep the organic materials in 70% ethanol.

Follow the same procedure for each section. Always clean the sieve before starting on another section of GI tract. Measure the empty weight and volume of each section.

Clean and dry the collected foreign materials, then measure the weight and volume (if possible). Plastic sheets are needed, and a four-digit precision scale is necessary for measuring micro plastics (from 1 mm to 5 mm in diameter). After measuring, label and keep all samples in a plastic bag.

Chemical pollutants represent a potential threat for sea turtles too. This is especially significant when the several large rivers that flow into different parts of the Mediterranean and its semi enclosed nature are taken into consideration. The presence of heavy metals in sea turtles has been studied in different parts of the Mediterranean Sea. Most of the concentration values were below toxicity levels, apart from the north Adriatic (Franzellitti et al., 2004) and the sea off southern Turkey (Kaska et al., 2004).

Recently, Cortes-Gomez et al. (2017) reviewed the metal concentrations revealed in 58 studies among sea turtle species. They summarised the results and reported that the accumulation of pollutants varies between species, the geographic locations and their life-stages. Ross et al. (2017) also reviewed the toxic metal contamination in sea turtle tissues from 95 studies and remarked on the implications for human health.

Stranded sea turtles are extremely useful for molecular studies, stable isotope analysis and skeletochronology and should be monitored regularly. Carapace length is a parameter commonly recorded from most stranded and rehabilitated turtles. Although stranded individuals are certainly a biased sample, they offer the most cost-effective method for collecting information about size distribution in foraging grounds.

2.4.6. Habitat use: stable isotope analysis

Stable isotope analysis (Carbon (^{13}C), Nitrogen (^{15}N) and Sulphur (^{34}S)) offers an inexpensive method for mass monitoring. The Mediterranean Sea is subdivided into a number of isotopically distinct sub-basins (Cardona et al., 2014), which offers a good opportunity to use stable isotopes as habitat markers both for loggerhead and green turtles (Zbinden et al., 2011; Cardona et al., 2014). Regular collections of tissue samples from nesting females will enable the identification of the foraging grounds used by the females nesting at each major site.

The first approach is the collection of tissue samples from adult satellite tagged turtles, tagged at their nesting beaches, and the use of the stable isotope ratios in these samples to characterize the foraging grounds of the turtles (Zbinden et al., 2011).

The second approach is the collection of tissue samples of adults and juveniles captured at their foraging grounds and use the stable isotope ratios to characterize them. This approach assures a large sample size from most areas, but there is no way to discriminate between transient and resident individuals, which will reduce the spatial accuracy of the data. The stable isotope ratios of satellite tracked turtles are also useful for identifying potentially transient individuals.

The third approach is the use of stable isotope ratios in potential prey from different foraging grounds to characterize them. This is necessary in order to understand the sources of variability among foraging grounds and to make sure that differences in the stable isotope ratios of turtles are because of differences in the isotopic baseline and not because of variances in diet. However, to derive stable isotope ratios in turtle tissues from those of their potential prey is not straightforward, even if prey-to-predator discrimination factors are known.

Tissue selection is critical for stable isotope analysis, as diet-to-predator discrimination factors are tissue dependent (Seminoff et al., 2006; Reich et al., 2008; Vander Zanden et al., 2012). Skin is probably the best option, as can be sampled easily from both dead and alive individuals and integrates diet over several months. However, collecting skin samples from most females is unlikely at most nesting beaches due to logistical constraints.

Sampling dead hatchlings is easier and less intrusive, but the probability of finding a dead hatchling increases with clutch size and hence this approach may bias the sample in favour of the females using the most productive foraging grounds, as they lay more eggs (Cardona et al., 2014). Egg sampling offers an alternative to avoid such a bias, but this means that each nest has to be excavated once discovered. Furthermore, the methods need to be improved to infer stable isotope ratios in female skin from those in an egg.

2.4.6.1. Sample Collection for Stable Isotope Analyses

The most common stable isotope sampling tissues are blood, carapace and skin from live turtles. Bone samples from dead turtles also contains important information. Each tissue may contain different information about their life cycle.

The volume of a sample needed for stable isotope analyses is minimal. Samples of 0.5g to 2.0g samples are sufficient.

To collect blood, follow the same procedure as given previously.

If samples are to be collected from other tissues, bear in mind that all samples must be collected from the same part of each animal. Tissues collected from different parts of the animals (e.g. a skin sample from the proximal part of the front flipper from one turtle and a skin sample from another's rear flipper) may provide different information and as a result the study samples will not be homogenous.

Sampling from the skin: Begin by cleaning the sampling area. Gently remove any epibionts and algae and rinse with water. Using a 6 mm biopsy punch is an easy way to obtain a skin sample. If using a scalpel, restrain and immobilize the turtle and use forceps to facilitate sampling. Place the samples in cryovials or Teflon bags and store, frozen to at least -20°C, until analysis.

Sampling from the carapace: There are two methods for collecting scute samples: cutting a small keratein with biopsy punch or a scalpel, and shaving. If the turtle is large, use a biopsy punch or scalpel to sample the scute. In this way, it is possible to collect different layers of keratin tissue. Be careful when using a scalpel, as the blade can break during sampling.

Start by cleaning the sampling area of algae, sand any other materials. Gently shave the top layer then rinse with distilled water, if possible. An 1X1 cm scute sample is usually enough for analysis. Try to reach the white epidermal tissue under the keratin layer. After sampling, remove the white epidermal tissue from the scute. Rinse the sample with ethanol and air dry it to facilitate removing the tissue. Place the samples in cryovials or Teflon bags and store, frozen to at least -20°C, until analysis.

If the samples are from a juvenile turtle, collect samples with shaving the scute, as the keratin layer is very thin, especially in green turtles. Clean and rinse the sampling area, then start shaving an entire scute using a knife (the 5th ventral scute is suitable for this procedure). Approximately 2.00 mm of the keratin can be shaved. Use a wind shield (e.g. umbrella) while shaving. Place the samples in cryovials or Teflon bags and store, frozen to at least -20°C, until analysis.

<u>Required Equipment</u>

- Biopsy punch
- Scalpel
- Blade (for scratching)
- Vials (for sample storage)
- Teflon bags (for sample storage)
- Ethanol 70%
- 21g needle and syringe/vacutainer (for sampling blood)
- Heparinized blood tubes
- Centrifuge (for separating blood cells from the plasma)
- Vials and cryo tubes

2.4.7. Contributions from fisheries

Fishing activities are one of the main threats to sea turtles, as they can be caught as bycatch in the various fishing gears. Then again, collaborating with fishermen can be an important monitoring tool. Such partnerships allow researchers to collect data from inaccessible areas, especially from pelagic areas. When limitations such as time, human resources, and budget and so on are taken into account, collecting data from oceanic areas is invariably difficult but the following information can be gathered from fishing operations:

- Distribution ranges in marine habitats
- Demography
- Sex ratio in marine habitats
- Tag return
- Seasonality of marine habitats
- Sampling tissues (e.g. blood, skin, scute)
- Health assessment

Researchers are able to collect data on-board during fishing operations. In addition, fishermen may provide important information by self-sampling without the assistance of a researcher. There are also technologies available for *citizen scientists*, such as smart phone applications for collecting data on an entangled or a stranded animal. Smart phones can also be provided to the fishermen to encourage their involvement in monitoring projects. Nevertheless, with or without new technologies, fishermen can collect the following data:

- Entangled sea turtle species
- GPS location
- CCL measurement
- Tag return information
- Tagging
- Photograph of entangled/stranded turtles

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E. Guidelines for monitoring non-indigenous species (NIS)

<u>Table of Contents</u>

1. Background

The Ecosystem Approach (EcAp) process was elucidated in 2008 at the 15th Meeting of the Contracting Parties to the Barcelona Convention, in Decision IG. 17/6, with the vision of "A healthy Mediterranean with marine and coastal ecosystems that are productive and biologically diverse for the benefit of present and future generations", along with an Ecosystem Approach Roadmap, aiming to achieve this vision. Subsequently, the Parties agreed on strategic goals to achieve the Ecosystem Approach vision, on 11 Ecological Objectives (EOs), and on matching Good Environmental Status (GES) descriptions, targets and indicators, including EO 2 (Non-indigenous species).

At their 19th Ordinary Meeting (COP 19, Athens, Greece, 9-12 February 2016), the Contracting Parties (CPs) to the Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention) adopted the Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP) which describes the strategy, themes, and products that the Contracting Parties are aiming to deliver, through collaborative efforts in the framework of the Mediterranean Action Plan(MAP) , during the second cycle of the implementation of the Ecosystem Approach Process in 2016-2021.

The overarching principles guiding the development of the IMAP include (i) adequacy; (ii) coordination and coherence; (iii) data architecture and interoperability based on common parameters; (iv) concept of adaptive monitoring; (v) risk-based approach to monitoring and assessment, and (v) the precautionary principle, in addition to the overall aim of integration.

Data and information are gathered through integrated monitoring activities on the national level and shared in a manner that creates a compatible, shared regional pool of data, usable by each Contracting Party. The IMAP information system will ensure the establishment of the regional pool of data and will allow the production of common indicator assessment reports in an integrated manner, following the monitoring specifics and data provided, which ensures comparability across the Mediterranean region. Integration is achieved through IMAP both at monitoring level, through an integrated monitoring system, following common principles and undertaken in a coordinated manner, and at assessment level, with the overall aim to assess the overall status of the marine and coastal environment.

The common indicators are the backbone of IMAP which covers 11 ecological objectives including the non-indigenous species (EO2), Citing UNEP/MAP (2017):

'In the context of the IMAP, a common indicator is an indicator that summarizes data into a simple, standardized, and communicable figure and is ideally applicable in the whole Mediterranean basin, or at least on the level of sub-regions, and is monitored by all Contracting Parties. A common indicator is able to give an indication of the degree of threat or change in the marine ecosystem and can deliver valuable information to decision makers.'

1.1 Definitions

The following definitions have been extracted from the Decision IG.22/7 (Barcelona Convention, COP19, 2016) entitled ''Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria'' and from the Joint Research Centre (JRC) guidance document on the MSFD Descriptor 2 (Non-indigenous species), citable as Olenin et al. (2010).

Non-indigenous species (NIS; synonyms: alien, exotic, non-native, allochthonous) are species, subspecies or lower taxa introduced outside of their natural range (past or present) and outside of their natural dispersal potential. This includes any part, gamete or propagule of such species that might survive and subsequently reproduce. Their presence in the given region is due to intentional or unintentional introduction resulting from human activities. Natural shifts in distribution ranges (e.g. due to climate change or dispersal by ocean currents) do not qualify a species as a NIS. However, secondary introductions of NIS from the area(s) of their first arrival could occur without human involvement due to spread by natural means.

Invasive alien species (IAS) are a subset of established NIS which have spread, are spreading or have demonstrated their potential to spread elsewhere, and have an adverse effect on biological diversity, ecosystem functioning, socio-economic values and/or human health in invaded regions. Species of unknown origin which cannot be ascribed as being native or alien are termed cryptogenic species. They also may demonstrate invasive characteristics and should be included in IAS assessments.

The key term "…levels that do not adversely alter the ecosystems" is described as the absence or minimal level of "biological pollution". The latter is defined as the impact of IAS at a level that disturbs environmental quality by effects on: an individual (internal biological pollution by parasites or pathogens), a population (by genetic change, i.e. hybridization), a community (by structural shift), a habitat (by modification of physical-chemical conditions) or an ecosystem (by alteration of energy flow and organic material cycling). The biological and ecological effects of biopollution may also cause adverse economic consequences.

1.2 Legislative framework outside EcAp

The CBD's (Convention on Biological Diversity) Strategic Plan for Biodiversity 2011-2020 includes twenty measurable Aichi Biodiversity Targets, which need to be met by 2020, including Target 9 which refers to NIS: 'By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment.'

COP Decision VI/23 includes guiding principles for the prevention, introduction and mitigation of impacts of alien species that threaten ecosystems, habitats or species9. Guiding principle 5 on Research and monitoring recognizes that these are required not only to develop an adequate knowledge base to address the problem but are also key to early detection of new invasive alien species.

Monitoring should include both targeted and general surveys, and benefit from the involvement of other sectors, including local communities. Research on an invasive alien species should include a thorough identification of the invasive species and should document: (a) the history and ecology of invasion (origin, pathways and time-period); (b) the biological characteristics of the invasive alien species; and (c) the associated impacts at the ecosystem, species and genetic level and also social and economic impacts, and how they change over time.

The European Union's Marine Strategy Framework Directive (MSFD) is a wide-ranging framework directive (2008/56/EC) with the overall objective of achieving or maintaining Good Environmental Status (GES) in Europe's seas by 2020 (MSFD, 2008). Eleven high level qualitative Descriptors of GES have been defined in Annex I of the MSFD, including Descriptor 2, for which GES has been defined as 'Non-Indigenous Species introduced by human activities are at levels that do not adversely alter the ecosystem.' Currently, the first six-year cycle of the MSFD is nearing completion, with EU Member States having submitted to the EU Commission their respective Programme of Measures (PoM) prior to their eventual implementation, following the collection of monitoring data for different Descriptors.
EU Regulation 1143/2014 lists the Invasive Alien Species (IAS) of Union Concern which should be the target or management measures and in which no commercial trade is allowed. Currently, this Regulation lists only terrestrial and freshwater species, and not marine ones.
Parties to the Bern Convention are required to Parties "to strictly control the introduction of non-native species" (Article 11.2.b). The European Strategy on Invasive Alien Species adopted under the framework of the Convention similarly addresses research and monitoring10. Monitoring that is systematic helps build an understanding of the ecological, distribution, patterns of spread and responses of IAS to management.

1.3 Scope and introduction to EcAp Common Indicator 6

The scope of this document is to elucidate the monitoring guidelines to address the EcAp Common Indicator 6: "Trends in abundance, temporal occurrence and spatial distribution of non- indigenous species, particularly invasive non-indigenous species, notably in risk areas in relation to the main vectors and pathways of spreading of such species".
This Common Indicator was selected by the February 2014 Integrated Correspondence Group on GES and Targets (Integrated CorGest) of the EcAp process of the Barcelona Convention from the integrated list of indicators adopted in the 18th Conference of the Parties (COP18), as a basis of a common

monitoring programme for the Mediterranean in relation to non-indigenous species, being preferred over other Common Indicators for Ecological Objective (EO) 2 (Non-indigenous species), such as the 'Ratio between non-indigenous invasive species and native species in some well-studied taxonomic groups.'

Common Indicator 6 is a trend indicator, whose main objective is to establish reliable, long-term data-sets as a first step of monitoring. In order for this trend indicator to become operational, at least two years of relevant data are necessary, in order to allow a minimal comparison of two annual datasets. In the absence of relevant pre-application (of the trend indicator) data, it is advised to deploy a two-year dataset collected after the optimisation of the indicator.

Although the GES for EO2 has not yet been fully elucidated by Contracting Parties, with respect to Non-Indigenous species, UNEP/MAP (2014) establishes the following aspirations:

(i) that no new non-indigenous species are introduced, and
(ii) that the number and composition of non-indigenous species have decreased to such a level where only non-indigenous species which had previously settled at a location are present, i.e. a reference level indicating that the number of non-indigenous species has remained the same in the period of three successive years, assuming that the eradication of established marine NIS is virtually impossible.

1.4. Aims and objectives

The main aim of this document is to provide guidance to environmental management practitioners (e.g. environmental authority representatives, researchers, students, Marine Protected Area [MPA] representatives) on field methodologies for monitoring Non-Indigenous Species (NIS) in MPAs and in identified hotspots. This provision of guidance is pursuant to enabling the same practitioners to achieving the goals of EcAp Common Indicator 6, by reviewing recognised good practices in the field of NIS monitoring protocols.

2. Monitoring protocol

2.1 Rationale and strategy

Two potential metrics/attributes of the Common Indicator 6 identified within UNEP/MAP (2014) are the following:

(i) Abundance of non-indigenous species
(ii) Temporal occurrence and spatial distribution of non-indigenous species

(i) It is widely recognised that the collection of abundance monitoring data is an expensive process. It is thus recommended to focus monitoring efforts on the recording of all NIS in a particular area – i.e. on the compilation of site-specific NIS inventories. The collection of abundance monitoring data might only be justified in cases of a species exhibiting abrupt spreading beyond a pre-defined threshold. Given the broad geographical range of monitored areas within different Contracting Parties, it is recommended that these thresholds are calculated as a fraction or percentage of the total monitored coastline, rather than as an absolute length of coastline. A relevant threshold example could be the spread of a NIS within a coastal stretch exceeding 5% of the total national coastal extent, or the doubling of the number of coastal monitoring stations at which a NIS has been reported.

(ii) To monitor the trend indicator of non-indigenous species two parameters [A] and [B] should be calculated on a yearly basis. Parameter [A] provides an indication of the introductions of new species (in comparison with the prior year), and parameter [B] gives an indication of the increase or decrease of the total number of non-indigenous species, computed as follows:

[A]: The number of non-indigenous species at T_n (year of reporting) that was not present at T_{n-1} (previous year). To calculate this parameter, the non-indigenous species lists of both years are compared to check which species were recorded in T_n, but were not recorded in T_{n-1} regardless of whether or not this species was present in years antecedent to T_{n-1}. To calculate this parameter, the total number of non-indigenous species is used in the comparison (although species names should also be listed).

[B]: The number of non-indigenous species at T_n minus the number of non-indigenous species at T_{n-1}.

Trends in both [A] and [B] should be monitored to develop the best management plan for non-indigenous species in an area.

2.2 Spatial and temporal considerations (the 'Where' and the 'When')

It is recommended that NIS surveys are conducted within both 'hotspots' areas (e.g. ports and their surrounding areas, docks, marinas, aquaculture installations, heated power plant effluents sites, offshore structures) and within marine areas subject to some form of environmental management, most notably Marine Protected Areas (MPAs).

'Hotspots' are defined as the most feasible entry/introduction points for NIS by virtue of:

(i) a preliminary desk study which identifies particular site-specific features (e.g. a harbour frequented by a considerable number of vessels) or

(ii) an elevated number of NIS already established within the confines of the same hotspot.

Typically, hotspots would include site typologies such as harbours, ports, yacht marinas, mariculture cages, offshore structures and thermal effluent discharge locations. Sites not necessarily in close proximity to these 'conventional' hotspots could also be considered within this same category, including locations subject to intense anchoring pressure during the tourist season.

In terms of NIS 'hotspots', UNEP/MAP (2014) recommends that NIS monitoring is conducted for at least two hotspot locations per potential introduction pathway, most notably commercial shipping, recreational boating and aquaculture. The same report provides guidance in the form of criteria, which should be applied when selecting candidate hotspot locations, as follows:

- Past research has shown them to be hotspots for non-indigenous species that can be transported with the transport vector concerned;
- The species communities at the two locations do not directly influence each other;
- Vulnerable areas with prospects for 'inoculation' or invasion by new introductions.

In terms of MPAs, a minimum of two sampling stations per MPA are recommended, with the two stations being located within different management zones within the same MPA. In terms of the specific positioning of the two NIS monitoring stations within each MPA, it is recommended to ensure a high degree of geographical and ecological representativity. This can be ensured in a variety of ways, including:

(a) opting for a minimum threshold of physical distance between the two sampling stations, expressed as a percentage of the total lateral extent of the MPA in question (e.g. the distance between the two sampling stations should not be inferior to 25% of the total lateral extent of the MPA);

(b) opting for sampling stations dominated by different marine biocoenoses (e.g. algal-dominated rocky reef versus seagrass meadow);

(c) opting for sampling stations incorporated within anthropogenic or ecological features of interest, with potential candidates including wrecks (which are considered as promoting the establishment of NIS – e.g. Bariche [2012]), a benthic area heavily impacted by anchoring or a sea urchin barren.

The exact geographical location of each selected sampling station in both hotspots and MPAs should be recorded through GPS coordinates, so as to enable consistent sampling on successive occasions.

In terms of sampling frequency, it is recommended that hotspots are monitored on a bi-annual/six-monthly frequency, so as to cover both spring and autumn seasons, with the same monitoring survey being conducted after three years.

MPAs should be monitored on an annual basis (preferably in spring), given that the rate of introduction of new NIS within MPAs is expected to be lower than that observed within hotspots, such that the latter sites should be sampled with a higher intensity. The rationale behind the preference for the spring season for monitoring purposes is that recruitment in most marine species takes places during this season, and thus conducting monitoring surveys in spring allows for the collection of different NIS life stages which only occur during this time of the year.

The following table summarises the recommended spatial and temporal recommended dimensions of the NIS monitoring:

Sampling location typology	**Recommended number of sampling stations**	**Recommended sampling frequency**
'Hotspots'	Two per NIS introduction pathway	Bi-annual/six-monthly
Marine Protected Areas (MPAs)	At least two per MPA	Annual

2.3 Procedures (the 'Which' and 'How')

Which NIS to focus upon within the trend analyses is one of the most important considerations to make. The trend indicator (2.1ii), in fact, hinges on the compilation of a preliminary inventory of NIS present within a monitored marine area, which will then also feed into attribute/metric 2.1i. The compilation of this baseline NIS list will also, in turn, allow the identification of reference conditions and thus facilitate a better definition of GES for EO2. This first NIS inventory can be compiled through the exclusive or mixed deployment of any of the following tools:

(a) **Rapid Assessment Survey**. According to Lehtiniemi et al. (2015), rapid assessment is 'a synoptic assessment, which is often undertaken as a matter of urgency, in the shortest time frame possible to produce reliable and applicable results for its defined purpose. Protocols for rapid assessment of marine and coastal biological diversity are available (e.g. UNEP/CBD/SBSTTA/8/INF/13 – Pedersen et al., 2005). Rapid assessment monitoring for targeted species enables direct reporting to management when a notable species is encountered and the 'field' work can be undertaken by a small group of experts. The method is cost-effective and relevant when prompt management response is sought, but unsuitable for detection of newly arrived introductions;

(b) **Literature review**, specifically of recently-published (preferably not earlier than 2010) national censuses or inventories of recorded NIS. For EU Member States, the MSFD IA (Initial Assessment) reports for Descriptor 2 could hold useful relevant information, as well as a number of international and regional (European or Mediterranean basin-scale) databases and lists. These include the European Alien Species Information Network (EASIN) developed by the Joint Research Centre of the European Commission, which facilitates the exploration of non-indigenous species information in Europe (and the entire Mediterranean), from distributed resources through a network of interoperable web services, following internationally recognized standards and protocols. Additional global relevant databases include the CABI Invasive Species Compendium, the GISD (IUCN Invasive Species Specialist Group and IUCN Global Invasive Species Database) and FISHBASE, whilst additional databases of regional interest include DAISIE (Delivering Alien Invasive Species Inventories for Europe), the CIESM Exotic Species Atlas linked with NIS base, the MAMIAS Database from the Specially Protected Areas Regional Activity Centre (SPA/RAC) of the UNEP/MAP Barcelona Convention and the ESENIAS East and South European Network for Invasive Alien Species. Regional data portal on invasive alien species (IAS) in East and South Europe.

(c) **Citizen science**. With rigorous quality control in place, national and regional citizen science campaigns are ideal for NIS monitoring purposes. Members of local communities, due to their broad geographic distribution and familiarity with their natural environment, can in fact, be of great help to track invasive species in both terrestrial and aquatic systems (Delaney et al., 2008). A renewed drive to identify components of the natural world, through 'bioblitz' events organised round the globe, is bolstering the interaction between formal scientists and informal/citizen ones, also through the availability of low-budget underwater photography and video-capture hardware on the market. An example of a national citizen science campaign is Spot the Alien Fish (www.aliensmalta.eu) one, targeting fish NIS in the Maltese Islands, whilst a number of additional citizen science campaigns operate on.

Within hotspots, a two-pronged monitoring approach is recommended, namely:

(i) Rapid Assessment Survey, as optimised for NIS monitoring within hotspots in Minchin (2007) and in UNEP/MAP (2014). These surveys are conducted by a team of marine species experts spending a specified time period (ideally, this is standardised to ensure uniformity, with a duration of 30 minutes considered to be a feasible one for each individual survey) at the survey site (preferably through SCUBA diving, but possibly even through snorkelling in very shallow areas) and identifying species by observation of artificial substrates such as jetties and wharves, pontoons, long-standing buoys and other artificial structures such as fish-farm cages. A site master records the scientists, findings and abundance of species at each site. Samples of specimens may also be taken back to the lab, where species identification is confirmed, through ex situ analyses involving dissection, microscopic examination and liaison with reputable taxonomists of a pan-Mediterranean profile. This is especially feasible for taxonomically-challenging groups such as sponges, hydroids, serpulids, bryozoans and ascidians. In order to further assist in taxonomic identification efforts within the targeted taxa, samples of recorded species should be preserved in absolute, non-denatured ethanol for subsequent molecular analyses. The basic equipment necessary to conduct this monitoring survey includes underwater photographic and/or video cameras, preferably supplemented by the provisions of high levels of artificial light (e.g. through the provision of strobes or basic flash) and underwater data recording facilities, which might include an underwater slate and pencil, or a laminated notebook, per SCUBA diver.

(ii) Scraping technique. This is to be deployed along vertical transects running from the surface of the monitored artificial structure hosting the fouling assemblage down to the foot of the same structure, with sampling stations being placed at a minimum of three different depths along the same transect. The scraping protocol was developed within CIESM's PORTAL programme (Galil, 2008), which in turn was based on the CRIMP methods first described by Hewitt & Martin (1996) and later by Hewitt & Martin (2001). It involves the collection of the fouling community enclosed within a quadrat of standard dimensions (commonly, 50cm x 50cm) through scraping by means of appropriate utensils (e.g. hammer and chisel), within a fine-mesh bag, followed by ex situ, laboratory analyses and identification. Once on land, the collected samples should be preserved by placing the fine-mesh bag directly in a five-litre bucket where its contents are left to soak in non-denatured ethanol (at least 70%) prior to laboratory examination. Different preservatives other than ethanol might need to be deployed for taxa such as ascidians, for which a formaldehyde:seawater mixture is preferred. Caution should be applied when handling formaldehyde given its highly corrosive and carcinogenic nature.

Figure 1 illustrates the standard 50cmx50cm quadrat normally deployed during scraping exercises within fouling communities.

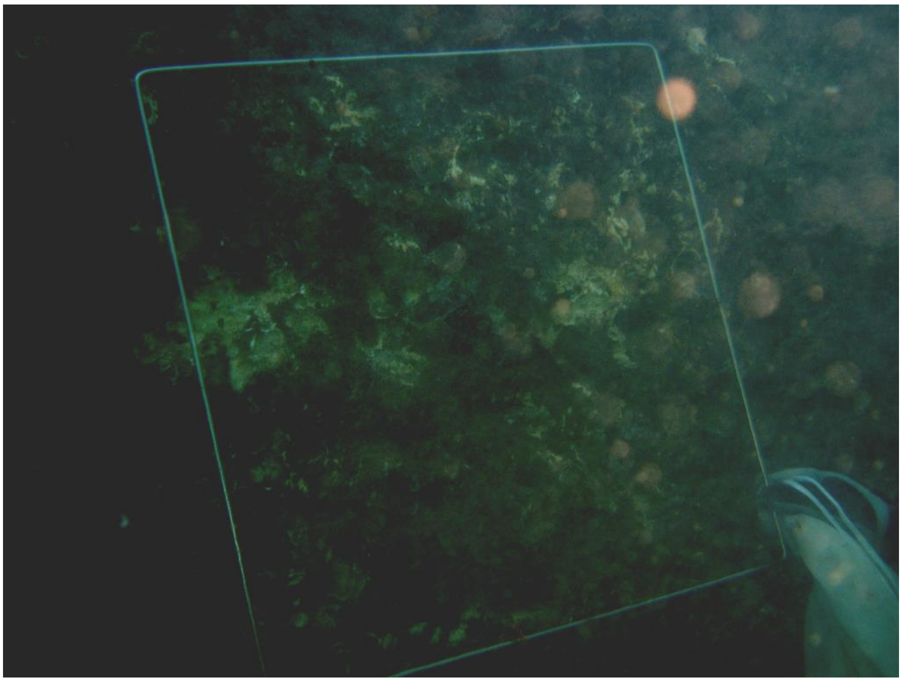

Figure 1 – 50cmx50cm quadrat deployed during scraping exercises within fouling communities (credits for photo: A. Deidun).

Within MPAs, the monitoring protocol for NIS have been developed by the IUCN and is elucidated in Otero (2013). Linear transects having an individual length of 100m, perpendicular to the shoreline and representative of the habitats, depth ranges and substrates within the MPAs are identified. Three replicate and comparable transects at each MPA sampling station are deployed, with a minimum distance of 10m between each transect. Ideally, the linear transect is laid out in the field through the use of a measuring tape of adequate length, which is secured on the seabed at both ends through the use of extra weights.

The location of each transect is identified by GPS coordinates for latitude and longitude to ensure faithful reproduceability in future occasions of the conducted monitoring. Non-indigenous species encountered up to five meters on either side of transect are recorded, counted and geo-referenced. Figure 2 illustrates the field conduction of the prescribed monitoring protocol within MPAs.

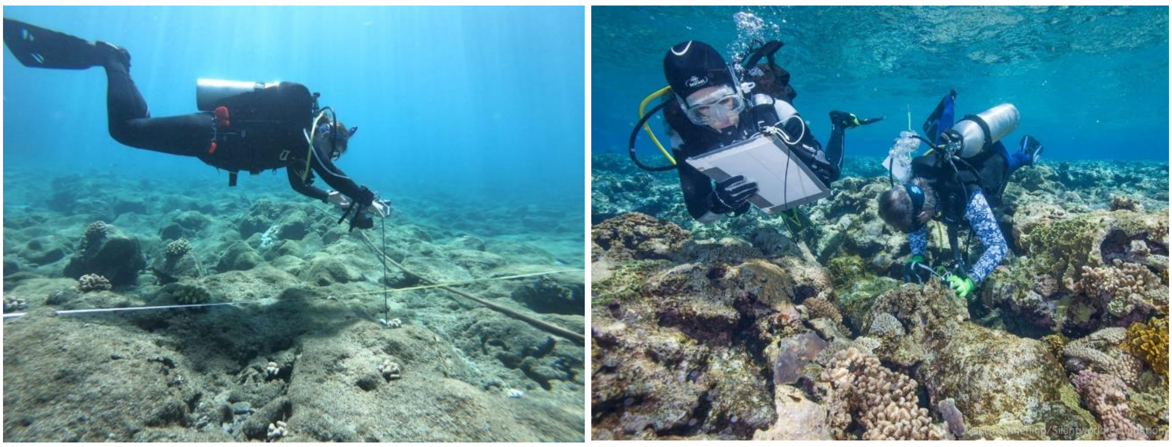

Figure 2 – Field conduction of the proposed monitoring protocol within MPAs (credits for photos: http://blog.owuscholarship.org/).

The water depth at which different NIS species are recorded during RAS or at which scraping samples are collected should be recorded. SCUBA divers must thus be equipped with water depth gauges to be able to achieve this requisite. Voucher specimens of first records should be retained within catalogued collections for reference purposes.

Additional, complementary data which should be collected for **both hotspots and MPA**s on a non-mandatory basis include:

(a) Semi-quantitative estimates of abundance of both (i.e. native and non-native) community components, through the deployment of different techniques for different taxonomic groups. For instance,

(i) for fish, direct counting for a fixed (e.g. 10-15 minutes at each site) span of time within a visual census could be deployed;
(ii) for benthic macroalgae, direct counting of clusters of the same species, followed by an estimation of the Braun-Blanquet cover index for a standard number of clusters (e.g. 3) of the same macroalgal species could be performed. A similar approach would be useful for quantifying sessile, encrusting invertebrates present in the area. Alternatively, the CARLIT index, adopted within the Water Framework Directive (WFD) and the MSFD, could be quantified;
(iii) individuals sessile and slow-moving non-encrusting invertebrates (e.g. gastropods) can be counted directly over a pre-determined time span (e.g. 10-15 minutes) or within a pre-determined spatial area (e.g. 5mx5m benthic area).
(b) Values for salient water biogeochemical parameters, including water column temperature, salinity and dissolved oxygen content, should be recorded, where possible.
Collection of ancillary socio-economic metrics, through:
(c) Preliminary observations of tangible impacts of the recorded NIS on native species, also through semi-quantitative (and probably arbitrary) indices of impact intensity on native species, potentially including broad impact categories ranging from 'High' to 'Low';

(d) Assessment and identification of potential introduction pathways for each recorded NIS.

Assessment of potential introduction pathways should take into consideration ongoing developments from the pathway assessment exercise by the IUCN-Species Survival Commission-Invasive Species Specialist Group on pathway terminology, classification and analysis of pathway data (http://www.cbd.int/doc/meetings/cop/cop-12/information/cop-12-inf-10-en.pdf).

The salient features of every proposed NIS monitoring protocol for both invasion hotspots and MPAs are summarised in Table 1.

Table 1 - Summary table of salient features of the proposed NIS monitoring protocols for invasions hotspots and MPAs.

Monitored marine area typology	Monitoring parameter	Recommended monitoring methodology	Recommended equipment to be deployed during monitoring	Advantages of monitoring protocol	Limitations of monitoring protocol
NIS hotspots	Number/diversity of broader NIS community	Rapid Assessment Survey (RAS)	• Underwater photographic and/or video camera • Underwater slates or notebooks	Rapid and easy to apply	Requires taxonomic experts in the field; might overlook some cryptic NIS through non-observation; provides only semi-quantitative measures of abundance

	Number, abundance and density of native and non-native fouling community	Scraping technique	• Quadrat (e.g. 50cmx50cm) • Chisel and hammer • Fine-mesh bag • Five-litre buckets • Preservative (e.g. non-denatured ethanol)	Exhaustively records all species (both NIS and non-NIS) occurring in an area; provides abundance and density (quantitative data)	Destructive technique
MPAs	Number and abundance of NIS	Linear transect and visual census technique	• Underwater photographic and/or video camera • Measuring tape • Extra weight for securing both ends of measuring tape Underwater slates or notebooks	Rapid and easy to apply; allows analyses of trends in NIS abundance if conducted regularly in the same area	Requires taxonomic experts in the field; might overlook some cryptic NIS through non-observation; provides only semi-quantitative measures of abundance

2.4 Data analyses and interpretation

A positive or negative trend in [B] illustrates respectively an increase and a decrease in the total number of non-indigenous species in an area, which is a good trend indicator of non-indigenous species. One also needs to calculate [A] however as it is possible to have both a negative trend in [B], indicating a decrease in the total number of non-indigenous species, and a positive trend in [A] at the same time, indicating that management in the area is not sufficient yet. A positive trend in [A] ([A]>0) indicates that ―new species are introduced into the area and one should therefore investigate how and with which pathway they are introduced. If this concerns a pathway introduced by anthropogenic activities, one may focus management on that pathway. If the new non-indigenous species arrive by their natural distribution capacities, one may focus on back tracking the location of origin and focus management on that location.
Consequently, for all monitored stations, [A] at T_n = [A] at T_{n-1} = [A] at T_{n-2} = 0 and [B] at T_n = [B] at T_{n-1} = [B] at T_{n-2}, should indicate that no new non-indigenous species were introduced in the last three years, and that the number of non-indigenous species is decreased to a level where only settled (for at least three years) non-indigenous species are present.

3. Data handling policies

NIS and ancillary data collected on a national basis should be validated by an expert panel prior to it being submitted to a pan-Mediterranean, geo-referenced repository which can referenced by different user typologies (e.g. MPA managers, government environmental agencies, NGOs, research institutes). The MAMIAS database is a good candidate for such a repository, given its pan-Mediterranean nature, but unless this database is re-activated and its public access reinstated, alternative, relevant repositories should be availed of, including the EASIN, CIESM and GBIF ones. Protocols detailing how the NIS databases held within the selected final repository can be supplemented by citizen science reports being submitted by the public should be elucidated at a subsequent stage.

Field workers engaged in the deployment of the monitoring protocols must be confident they are recording most of the NIS species occurring in a particular area, in order to ensure a good quality of the data being recorded. UNEP/MAP (2014) states that the minimum threshold of the total NIS in an area which need to be recorded is that of 90% and that different statistical techniques exist for assessing progress towards achieving this. Further guidance to NIS monitoring practitioners should be provided in future on how to quantify statistically the fraction of total NIS occurring in an area which have been sampled.

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